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# LINEAR REMOVAL OF SIMPLE ORGANIC COMPOUNDS IN THE ACTIVATED SLUDGE PROCESS

Lial F. Tischler, et al

Texas University
Austin, Texas

January 1968

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# *Linear Removal of Simple Organic Compounds in the Activated Sludge Process*

CENTER FOR RESEARCH IN WATER RESOURCES
Environmental Health Engineering Research Laboratory
Civil Engineering Department

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CENTER FOR RESEARCH IN WATER RESOURCES

Environmental Health Engineering Research Laboratory

Department of Civil Engineering

# LINEAR REMOVAL OF SIMPLE ORGANIC COMPOUNDS IN THE ACTIVATED SLUDGE PROCESS

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U. S. Department of the Interior

Federal Water Pollution Control Administration

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January, 1968

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January 1968

## ABSTRACT

The objectives of this investigation were: (a) to demonstrate zero order removal of several simple organic compounds alone and in a mixture by the heterogeneous microbial population in activated sludge, (b) to observe the removal rates of these individual compounds in terms of the laboratory and field parameters in use today, (c) to show that the summation of the removals of chemical oxygen demand (COD) of each of the compounds alone, results in the overall COD removal of a treatment unit containing a mixture of the organics in the same initial concentrations, and (d) to observe the removal of these simple compounds by two types of microbial population, filamentous and flocculent.

To accomplish these objectives activated sludge was grown from a sewage seed in a laboratory-scale, continuous flow aeration unit. The sludge was acclimated to a mixture of the organic compounds, which provided the sole carbon source for the sludge to be studied.

After acclimation in the continuous unit, the sludge was transferred to batch units for the kinetic studies. The compounds to be studied were added to the batch units and their disappearance with time was observed. Two analytical techniques, one specific and one overall (COD),

were used to measure the concentration of each organic compound used in this investigation.

The results show that the simple organic compounds, glucose, phenol, and aniline were removed linearly under the conditions of the investigation. The data recorded for both the specific analytical method and COD indicated this zero order removal. The removal rate of the individual compounds in a unit in which all three compounds were mixed was the same as the rate found when each compound was tested alone. Variations in the initial concentration of the compounds had no effect on the removal rate of each of the compounds by a given sludge. The addition of two linearly removed compounds, glycine and acetate, to the three previously studied had no effect on the latter's removal rates.

In order to demonstrate that COD removals from a mixed waste are the summation of the COD removals of the components of the mixture, the COD concentration of the contents in the batch units containing one compound were added and compared to the COD in a batch unit which contained a mixture of the compounds in the same concentrations as in the individual units. The results show that for the compounds used, this assumption was valid. Similar results were also obtained using total organic carbon as an overall parameter.

The effects of organic loading and biological solids on the linear removal rates were also studied. The data obtained indicate that the filamentous sludge which predominates at high loading levels removes glucose and phenol at higher rates than the flocculent sludges

observed at low and intermediate organic loadings. Increasing the biological solids concentration results in an increase in the linear removal rates of the simple compounds.

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## SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| BOD | Biochemical Oxygen Demand |
| COD | Chemical Oxygen Demand |
| C | Concentration |
| E | Enzyme |
| K, k | Reaction Constants (base e) |
| $L_f$ | Organic Loading<br>lb COD/day·lb MLVSS |
| MLSS | Mixed Liquor Suspended Solids |
| $X_a$ | Biological Solids Concentration (MLVSS) |
| MLVSS | Mixed Liquor Volatile Suspended Solids |
| mμ | millimicrons |
| M | micromoles |
| mM | millimoles |
| N | Nitrogen |
| P | Phosphorus |
| R | Exponential Growth Rate, $hr^{-1}$ |
| S | Substrate Concentration, Substrate |
| TOC | Total Organic Carbon |
| v | Reaction Rates |

# Chapter I

## INTRODUCTION

At present, aerobic biological treatment of the organic industrial and domestic wastewaters produced by society seems to provide the most economical solution to the problem of liquid waste disposal. Biological waste treatment provides an effective means of preventing pollution of streams, lakes, and oceans. Design of these systems by sanitary engineers involves careful study of the many parameters which affect microbiological processes. However, much of the information concerning the biochemical and microbiological properties of the common treatment systems is contradictory, which points up the need for a careful examination of the basic biological principles involved in these systems.

Sanitary engineers commonly use biochemical oxygen demand (BOD) or chemical oxygen demand (COD) as convenient parameters for the estimation of the organic content of wastes, because the wastes which must be treated by these biological systems contain many different organic compounds. Most of these compounds may be categorized as carbohydrates, proteins, and lipids. However, in the case of many industrial wastes, more exotic compounds may be present. Obviously, it would be extremely difficult, and in some cases impossible, to estimate individually each of the compounds in a complex waste. Thus, an estimate of the COD and BOD concentrations provides convenient, although crude, parameters for the evaluation of treatability and for design of the overall system.

In order to understand the actual biochemical systems involved, it is desirable to observe biological treatment on the micro-scale. For

this reason, the removal of the simple sugars, amino acids, and fatty acids which make up the polysaccharides, proteins, and lipids, which in turn form the complex wastes, must be examined. Removal of various simple organic compounds was investigated to give a better understanding of the kinetics of the activated sludge process.

## 1-1 Objectives

The primary objectives of this research were: (a) to demonstrate zero order removals of several simple organic compounds alone and in a mixture by a mixed population activated sludge, (b) to observe the removal rates of these organics in terms of the laboratory and field parameters in use today, (c) to show that the summation of the COD removals of each of the compounds alone, results in the overall COD removal of a treatment unit containing a mixture of the organics in the same initial concentrations, and (d) to observe the removal of these simple compounds by two types of microbial population, filamentous and flocculent.

## 1-2 Scope

Relatively simple compounds, namely, glucose, phenol, and aniline were fed to activated sludge in continuous laboratory units. The sludge developed in these units was transferred into batch units for the kinetic studies. The compounds to be studied were then added to the batch units, both individually and mixed, and the removal of each compound with time was observed. The concentrations of these substrates were measured by using analytical methods which were specific for each compound. This

procedure made it possible to observe the removal of each compound when a mixture of the organic compounds was tested. Chemical oxygen demand by the dichromate method was also used to estimate the organic content of the samples and was compared to the estimates obtained from the other analytical techniques. Biochemical oxygen demand was used to determine how much degradable material remained after the batch treatment of the wastes. The initial concentrations of the organic compounds were varied, along with the concentration of microorganisms in the units, in order to change the organic load on the system and observe the effects on the removal kinetics of the simple substrates and the microbial population of the sludge.

In order to demonstrate that the removal of COD from a complex waste is the summation of the COD removals of the components of the mixture, the COD of the effluent of the batch units, each containing one organic compound, was added and compared to the COD in a batch unit which contained a mixture of the compounds in the same concentrations as in the individual units.

A single experimental run was made using five pure compounds to observe the effect of additional compounds on the removal characteristics of the three previously tested. The overall COD removal of a mixture of the five compounds was also observed and compared to the three compound system.

## Chapter 2

## LITERATURE REVIEW

### 2-1 General

The activated sludge process is essentially a continuous culture of aerobic and facultative microorganisms in which the waste being treated serves as the organic substrate. The required oxygen can be supplied to the microorganisms by various means, usually using air bubbles injected under turbulent conditions into the mass of microorganisms and liquid. (15)

Application of this process normally requires a solids-liquid separation after the aeration process, with subsequent return of the biological sludge to the aeration chamber. Many variations of the activated sludge process have been developed but all are essentially modifications of the conventional system, and the same biological processes occur in each. These modifications attempt to provide more economical and complete waste treatment by optimizing the various controlling factors of the microbiological processes.

These controlling factors are basically those which govern the growth and metabolism of any microbiological system. The most important of these factors, with respect to the biological system present in the activated sludge process, are: pH, temperature, and quantity and availibility of nutrients. In this investigation, both pH and temperature were held constant, therefore, these parameters will only be discussed briefly in this review when general principles of bacterial growth are covered.

Nutrients required for the cell include both organic and inorganic compounds. The inorganic compounds and trace organics required for optimum

microbial growth are usually present in high enough concentrations to insure maximum process efficiency. However, some industrial wastes are deficient in nitrogen and phosphorus and these elements must be added to obtain an efficient process. Based on studies of many nutritionally-deficient wastes, a BOD : N : P ratio of 100 : 5 : 1 is usually sufficient (15).

The organic nutrient which the bio-system uses for its source of carbon and energy for growth is the waste which the engineer wants to remove from the water to be treated. It is this organic nutrient which is of primary concern in this investigation. Inorganic and trace nutrients were supplied in excess to assure that they were not limiting during the study.

Organic nutrient quantity or concentration, when applied to activated sludge, is usually represented by organic loading. Organic loading is conveniently expressed as the mass of organic substrate (usually as BOD or COD), per unit mass of biological sludge, per unit time in the system. Many modifications of the activated sludge process are based primarily on variations of the organic loading, which are obtained by changing the detention time or biological solids concentration in the process.

One primary requisite of the activated sludge process is a sludge with good settling properties, which assures optimum solids separation from the treated effluent. Solids which are lost in the treated water negate the beneficial effects of treatment, since they add an organic load to the receiving body of water.

One of the major effects of varying the organic loading to the activated sludge process is the change in the settling characteristics of the bio-mass. The settleability of the biological sludge deteriorates at both extremes of organic loading, but is usually most severe at high loadings. At these loadings sludge bulking occurs. This sludge is comprised principally of filamentous organisms and generally settles very poorly due to the physical characteristics of these organisms. At very low loadings, such as those found in the extended aeration modification of activated sludge, a diffuse, finely divided bio-mass is formed which also settles poorly. Ford (17) found, using a domestic and two industrial wastes, that the optimum organic loading range for good settling floc was 0.2 - 0.7 lb BOD/day/lb solids. Values above or below this range will probably give a poor settling floc, with the higher values being the most critical. One objective of this investigation was to observe the effects on substrate removal of organic loadings outside this range as well as in it.

Dissolved oxygen is essential for the operation of this aerobic process. Various investigators have shown that the minimum permissible oxygen concentration in the aeration tank is 0.5 mg/l (30, 13) if waste treatment is to be effective. However, Ford (17) has shown the activated sludge microorganisms in the endogenous phase of growth can undergo prolonged periods of anaerobiosis with little adverse effect on their assimilative capacity.

## 2-2 Enzymes and Bacterial Growth

Many types of organisms are present in a well operated activated sludge process. In most cases, the predominant organisms and those responsible

for the removal of the majority of the organic waste are bacteria. For this reason this discussion will center on these organisms, although the biochemical principles cited will apply to all the organisms present in the system.

## Enzymes and Enzyme Kinetics

Enzymes are simple or conjugated proteins which catalyze the metabolic reactions essential to the activities of the cell (8). The protein nature of enzymes results in specificity for a particular reaction or class of reactions (10). At one time it was believed that the transport of organic substrate into the cell was a diffusion or osmotic process, but it is now known that the movement of substrates into the cell is accomplished by stereospecific permeation systems (9). These "permeases" are functionally specialized enzymes and are distinct from the metabolic enzymes of the cell (9). Clarke and Meadow (7) showed evidence of permease synthesis for citrate, acetate, fumarate, and succinate and hypothesized their location in the bacteria's osmotic barrier. Thus, all of the reactions which result in the removal of an organic compound or compounds from wastewater in the activated sludge process are enzyme catalyzed, and the factors which effect enzyme action will influence the substrate removal kinetics of the entire system.

Both pH and temperature are important factors in enzyme action. High and low values of pH tend to denature enzymes (10) and help to set the pH limits of the biological process. Temperature tends to increase enzyme activity of most enzymes up to a temperature of approximately 45$^{o}$C (10).

Above this temperature the protein is rapidly denatured, except in a few exceptional cases, notably the thermophilic bacteria.

The kinetics of enzyme catalysis have been studied extensively by biochemists. Before discussing these kinetics, a brief discussion of terms is desirable. Most rate expressions are of the form of exponential powers of the concentrations of the substances which affect the reaction rate (18). The numerical values of these powers are used to define the "order" of a reaction with respect to that particular component. Often one or more of the concentration factors remains constant, thus giving the reaction a "pseudo" or apparent order. This is particularly true in enzyme catalyzed systems, in which the enzyme concentration often has little effect on the overall reaction rate.

In the early 19th century, studies of enzymes showed that often the rate of an enzymatic reaction was not simply proportional to the concentrations of the reactants (26). Brown in 1902 developed the hypothesis of an enzyme-substrate complex to explain the action of the enzyme invertase on sucrose (26). The modern version of the theory commonly used to describe enzyme-substrate interaction has developed from this hypothesis.

This theory postulates that enzyme-substrate reactions occur in two well-defined steps: (1) the formation of an enzyme substrate complex

$$E + S \underset{K_2}{\overset{K_1}{\rightleftharpoons}} E \cdot S,$$

and (2) the decomposition of the complex into the enzyme plus the reaction product.

$$E \cdot S \xrightarrow{K_3} E + P$$

where E = Enzyme

S = Substrate

P = Product

k = Reaction constants.

Michaelis and others recognized that an enzyme catalyzed reaction involving varying substrate concentrations had two limiting cases (10). When the substrate concentration is low, most of the enzyme exists in the free state and the reaction rate is proportional to the concentration of substrate. At high substrate concentrations, the enzyme exists primarily as enzyme-substrate complex, and the reaction rate, which is controlled by the breakdown of the complex, is independent of substrate concentration (26), i. e., zero order with respect to the substrate.

The mathematical equation which describes this relationship is commonly called the Michaelis-Menten equation. The standard form of this equation is:

$$v = \frac{V\,[S]}{K_m + [S]}$$

where $v$ = reaction rate at substrate concentration $[S]$

$V$ = maximum reaction rate

$K_m$ = Michaelis constant, usually in moles/liter

It is evident from examination of this equation that it satisfies the two boundary conditions previously stated. Many modifications of this equation have been proposed to satisfy certain observed reaction rates, but

most predict exactly the same dependence of rate on substrate concentration (26). Various methods are available to linearize the Michaelis-Menten equation and thus render it more amenable to investigation of enzymatic reactions. Many enzyme-substrate systems have been checked by these methods, and in most cases have been shown to follow the Michaelis relationship (26). This behavior, zero order reaction at high substrate concentrations and first order at low concentrations, is very common for single substrates (26).

Bacterial Growth

Since the removal of organic substrates from a wastewater is directly related to bacterial growth, the principles of this growth are important in biological waste treatment. The stages of growth of a bacterial culture reflect the interactions between the cell population and the many environmental factors influencing growth (38).

As mentioned previously, the concentrations of inorganic and trace organic nutrients are important, particularly nitrogen and phosphorus. Hydrogen ion concentration is also important. However, the organisms present in the conventional activated sludge system can usually operate effectively in the pH range 6.0 - 9.0 (15). Temperature is also important, tending to increase the growth rate up to a certain limit, which is a function of the species of organism (8). For most waste systems, 30°C is the optimum although in certain cases much higher temperatures are effective (13).

A typical growth curve for a bacterial culture involves at least four principal phases: the lag phase, the exponential growth phase, the maximum stationary phase, and the death phase (38). The sanitary engineer is principally interested in the exponential growth and maximum stationary phases since it is here that the majority of substrate removal occurs. Actually, most of the soluble substrate is removed from solution during the exponential growth phase and lack of nutrients causes the occurrence of the stationary phase. During the stationary phase, any substrate stored in the cells is utilized.

During the exponential growth phase, the organisms are growing at a constant, maximum rate and the many metabolic reactions of growth can be considered constant (32). In this phase cell growth can be described by the exponential growth rate which is an overall rate of the steady-state system (32). It is not, as some people have suggested, governed by an overall, master reaction but rather can be shown to be a function of the rate and equilibrium constant of each of the consecutive reactions in the entire metabolic sequence (32).

Monod (32) has developed an equation which can be used to describe the effect of a limiting nutrient on the exponential growth rate. This equation is a hyperbolic function of the form:

$$R = R_k \cdot \frac{C}{C_1 + C}$$

where C = concentration of growth limiting nutrient

R = exponential growth rate

$R_k$ = maximum growth rate

$C_1$ = concentration of nutrient at which $R = \frac{R_k}{2}$

The similarity of this equation to the Michaelis-Menten equation is obvious, especially when the growth-limiting nutrient is the substrate concentration, S.

According to Monod (32), the $C_1$ value in this equation should be related to the dissociation constant of the enzyme in the first step of the breakdown of the nutrient. However, since $C_1$ is related to the whole sequence of metabolic reactions rather than one reaction in particular, its value might be expected to be considerably smaller than the Michaelis constant for the first enzyme reaction. Monod's studies indicate that, in fact, $C_1$ values are often very small and the exponential growth rates observed are commonly independent of substrate concentration (32).

Occasionally, more complex forms of exponential growth occur, often involving sequential exponential growth rates. These types of growth will be briefly discussed later in this review.

## 2.3 Metabolism of Simple Organic Compounds

### Pure Culture Studies

Much work has been done by microbiologists using single substrates and pure cultures of microorganisms. The only work reviewed below will be that which pertains to the types of compounds used in this investigation.

When Monod (32) developed his equation for growth, the exponential growth rate of *E. coli* on glucose was essentially independent of

glucose concentration above a concentration of 0.2 mMole. Hetling and Washington (24) ran batch studies using pure cultures of P. fluorescens and E. coli utilizing glucose, sodium acetate, and glutamic acid. Each test measured bacterial growth on one compound. It was found that growth rate could be related to substrate concentration by an equation similar to that used by Monod. Substrate concentration was measured by COD in these studies.

Several investigators have studied the bacterial oxidation of aromatic compounds. Kramer and Doetsch (25) studied the growth on various aromatics of three genera of microorganisms capable of utilizing phenol. None of the fourteen species studied could utilize aniline, indicating either toxic effects or lack of inducible permeases specific for this compound. Stanier (37) studied the oxidation of various aromatics by fluorescent Pseudomonads. He found that this genus of microorganism could adapt to the oxidation of many aromatic compounds. The results of this study also indicated that phenol was oxidized by a mechanism different from that for benzoate and benzaldehyde.

When the effect of growth of a pure culture on two or more simple organics has been studied, complicated growth patterns have sometimes been observed. The most common of these effects is diphasic or "diauxie" growth, first described by Monod (8). When E. coli was grown on two sugars, a pentose and a hexose, growth occurred in two exponential phases, separated by a lag phase. This was described as growth on one of the sugars, followed by adaptation to and growth on the second sugar when the first was exhausted.

Hamilton and Dawes (21, 22) have shown a similar effect using organic acids and glucose as substrates for P. aeruginosa. Other investigators have observed similar results with different substrates and organisms. This "diauxic" effect probably results from the growth on one compound using "constitutive" enzymes, followed by induction of enzymes for use of the second substrate when the first becomes growth limiting.

## Mixed Culture Studies

Sanitary engineers are principally interested in substrate removal by a heterogeneous culture of microorganisms which is commonly found in conventional biological treatment processes. Many investigators have studied the removal of simple compounds by mixed cultures. Placak and Ruchhoft (34) evaluated the biological utilization of 32 pure organic compounds. The results (34) indicated that substrate removal was a function of the specific substrate and not the class to which it belonged.

Wuhrmann (42) was one of the first investigators to show zero order removal of pure organic compounds by activated sludge. He demonstrated that removal rates of glucose, aspartic acid, tryptophan, and butyrate were independent of their concentrations. Peptone, a complex substrate, was removed exponentially. More recent data (43) demonstrated that the Michaelis constant for glucose is approximately 1 μMole, indicating that linear removal of glucose would normally occur at concentrations found in wastewaters.

Wilson (44) showed that phthalic acid was removed by zero order kinetics. Data from Bethlehem Steel (14) demonstrated that removal of

phenol by bacteria also followed zero order kinetics. Chambers, Tabak, and Kabler (6), using a stabilization pond inoculum, showed rapid phenol removal and limited oxidation of aniline. These data (6) demonstrate that the removal of phenol and aniline, two closely related compounds, is not necessarily carried out by the same enzyme system or bacteria. Data of Carlson and Polkowski (5) demonstrated that glycine removal by an acclimated activated sludge was independent of glycine concentrations greater than 8 mg/l.

Downing (11) investigated the removal of glucose and sodium acetate, both individually and mixed, by an activated sludge acclimated to both compounds. It was found that the rates of removal of each of the two substrates were unaffected by the presence of the other. The data indicated that possibly each substrate was utilized by a different organism. Data (4) obtained using a glucose-fed anaerobic digester also showed zero-order removal, verifying that this type of kinetics can occur in all types of biological treatment systems.

Zollinger, Busch, and Stumm (45) used glucose and benzoate substrates to demonstrate additive removals. Glucose and benzoate were both removed from solution at rates independent of their concentrations and neither compound affected the removal rate of the other. The results of a study (45) in which glucose and galactose were used showed that both substrates were removed linearly, but that no galactose removal was noted until after all of the glucose was oxidized.

This sequential removal was also observed by Gaudy, Komolrit, and Bhatla (20) using glucose and sorbitol substrates. It was found that

removal of glucose was preferential, even for sorbitol-acclimated sludge. The sequential removal shown in these two investigations is similar to the "diauxie" growth observed by Monod and others who used pure cultures of microorganisms. However, since microbial populations present in the conventional biological treatment processes include a vast number of many different types of organisms, it is doubtful that sequential effects are significant. In certain cases, notably those of shock loads, these effects may need to be considered.

## 2-4 Removal of Complex Substrates

The organic compounds found in wastewaters are usually of a very complex nature. In most cases it is impossible to determine the simple organic compounds which make up the complex wastes. Sewage consists principally of carbohydrates, proteins, and lipids. Numerous other types of organic compounds are also present in smaller quantities. Industrial wastes often contain unusual types of compounds, both complex and simple. Before these complex substances can be used as substrates by the microorganisms they must be broken down into the simple compounds of which they are composed. In these simple forms, they can then be metabolized by the organisms to provide energy for growth and carbon for cell material.

Many types of kinetic models have been used by sanitary engineers in their attempts to describe biological treatment processes. One of the most common of these is the Monod equation. The concentration of limiting nutrient is considered to be the BOD or COD in the wastewater. It has often been modified to consider the relationship between microorganism

concentration and organic loading. The standard form of the Monod equation is not directly applicable to continuous cultures and must be modified if it is to be used to describe these systems.

Many investigators have found that the Monod equation could be used to fit their data. Wilson (44) found the Monod relationship described removal of substrate on a rolling-tube trickling filter. Data obtained by Tench and Morton (39) also supported this type of formulation. The Michaelis constant obtained in the latter studies indicated that the removal of BOD from the waste under consideration would probably follow first order kinetics. Hartmann (23) also states that the Michaelis model can be used to describe activated sludge kinetics.

Another common kinetic model used for describing biological treatment is the two-phase system. Garrett and Sawyer (19) first showed that a two-phase, discontinuous function could be used to describe growth kinetics. McCabe and Eckenfelder (29) have shown its application to biological treatment process design. This method assumes that substrate removal during the exponential growth phase is zero order. At low substrate concentrations the organisms enter the declining growth phase and substrate removal is concentration dependent. This dependence can be expressed by the simple first order equation (12):

$$\frac{d\,S}{d\,t} = K_1\, X_a\, S$$

where $S$ = concentrations of substrate at any time $t$

$K_1$ = logarithmic removal rate

$X_a$ = biological solids (MLVSS)

This simple first order equation has often been used to describe substrate removal kinetics in biological waste treatment systems.

McCabe (28) used the two-phase theory to fit laboratory and field data. Popel (35) found that BOD data obtained on a laboratory trickling filter could be described by the two-phase theory.

One problem often encountered in the treatment of complex wastes is that the removal rates are often retardant. In these cases, the most readily assimilable compounds are removed first, and those compounds which remain are removed at lower rates. Several methods have been used to describe this retardant removal.

Eckenfelder (12) showed the use of a composite exponential of the form:

$$\frac{S}{S_o} = \frac{S_1}{S_o} \exp\ (-K_1\ X_a\ t) + \frac{S_2}{S_o} \exp\ (-K_2\ X_a\ t) + \ldots$$

where $S$ = BOD remaining at time t

$S_o$ = total initial BOD

$S_1$, $S_2$ = initial BOD concentrations of compounds 1, 2, ..., respectively

$X_a$ = biological solids (MLVSS)

$K_1$, $K_2$ = respective removal rate constants

and $S_o = S_1 + S_2 + \ldots\ldots\ldots\ldots\ldots$

This equation was used to describe BOD removal kinetics for a pulp and paper waste.

Other efforts to consider retardant removal rates include considering the reaction rate to vary with substrate concentration (16) and with time (33).

All of the previously discussed models have been applied with some success to the kinetics of biological waste treatment. None has been successfully applied to all of the situations which occur in the laboratory and field. Except for the Monod equation, none has any real theoretical significance. The Monod equation is similar to the Michaelis-Menten equation for enzyme-substrate interaction which describes many enzyme-substrate systems. However, these equations are for single substrates and it seems doubtful that they can be directly applied to a complex waste, using BOD or COD or a substrate parameter.

Wuhrmann (42) was the first to suggest that the non-linear removals commonly associated with complex wastes are actually a summation of a number of zero order removals of the simple compounds which make up the complex substrates. In this case, the actual substrate removal would be a discontinuous function (43), and the general form of the removal curve as measured by BOD or COD would depend upon the removal rates of the individual simple compounds forming the waste. Tischler (40) has shown mathematically that the summation of a series of linear reactions can approximate first and second order kinetics. The complex kinetics which are often observed for sewage and industrial wastes undergoing biological treatment may be explained by the facts that most of the complex organics must be broken down into simple compounds before the microorganisms can assimilate them and that pure compounds are removed linearly.

Mathematically, the two-phase approach and the Monod Equation are quite similar. Both predict the same limiting conditions, zero order substrate removal at high substrate concentrations and first order removal at low concentrations. The Monod or Michaelis-Menten formulation represents these conditions by using a continuous function in the form of a hyperbola, while the two-phase theory uses a discontinuous function as previously described. Obviously, the two-phase theory fails at the point of discontinuity since bacterial growth is a continuous phenomenon. However, the ease of computation of the constants for the two-phase formulation often makes up for this fault, since the constants in the Monod Equation are much more difficult to calculate. The other common formulations used to describe substrate removal are essentially exponential in form.

# Chapter 3

## EQUIPMENT AND EXPERIMENTAL PROCEDURES

The primary investigation involved the determination of the removal of simple organic compounds from solution by an acclimated activated sludge. The activated sludge was obtained from a laboratory scale, continuous flow, activated sludge unit. Batch units were used for the kinetics studies. The samples from the batch and continuous units were analyzed for carbon content, the specific substrates used, and mixed liquor suspended solids, both total and volatile.

### 3-1 Continuous Aeration Unit

The continuous unit which was used to acclimate the sludge was constructed of plexiglass and mounted on a movable table. The unit used had the aeration and settling chambers combined in a single unit, separated by an adjustable plexiglass baffle. This arrangement provides an upflow clarification tank, which has been found useful in avoiding the scale-up problem found in horizontal-flow clarifiers (3). An adjustable effluent wier was provided and the aeration tank volume could be adjusted from seven to twelve liters. Air was supplied to the unit by a fritted stone diffuser. A schematic diagram of the continuous unit is shown in Figure 3-1.

The air supply for this unit and the batch units came from a one hundred psi wall outlet adjusted to a pressure of approximately ten psi. The compressed air was passed through a bottle-water-trap to eliminate any foreign matter from the air stream and to saturate the air with water to lower evaporation losses. The continuous unit was fed from an 18-liter

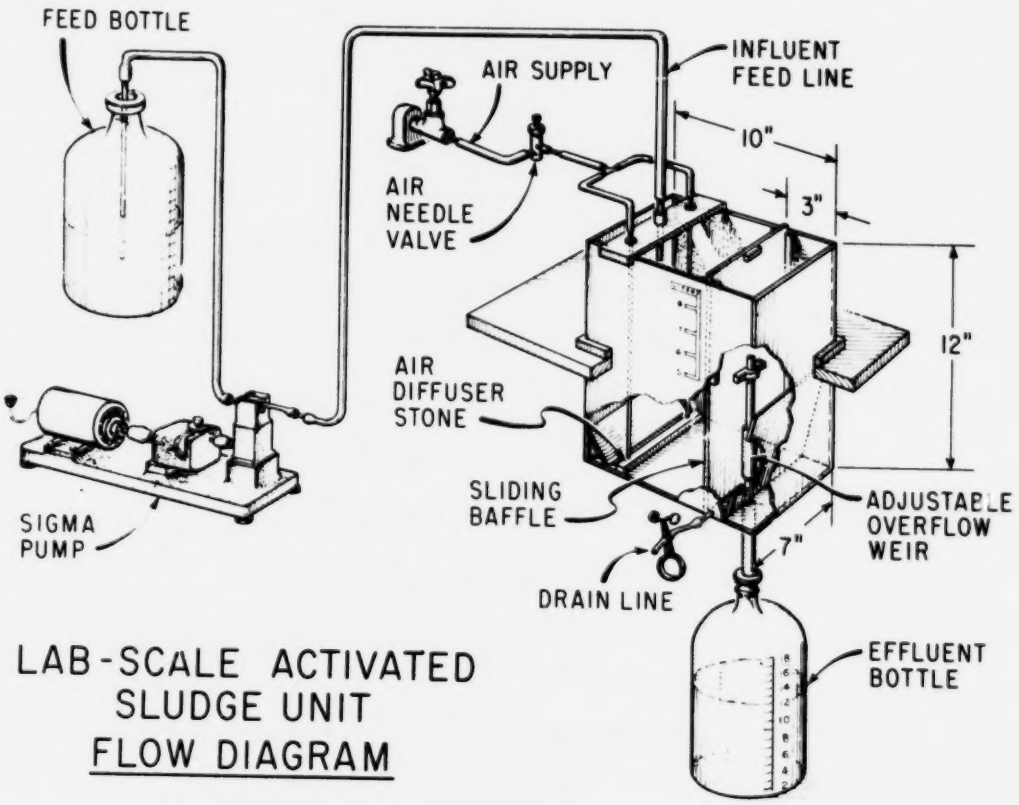
FEED BOTTLE
AIR SUPPLY
INFLUENT FEED LINE
10"
3"
AIR NEEDLE VALVE
12"
AIR DIFFUSER STONE
SLIDING BAFFLE
ADJUSTABLE OVERFLOW WEIR
7"
SIGMA PUMP
DRAIN LINE
EFFLUENT BOTTLE
LAB-SCALE ACTIVATED SLUDGE UNIT
FLOW DIAGRAM

Figure 3-1

carboy by means of a variable-speed sigmamotor pump. The flow rate was varied from 0.73 to 1.25 liters per hour to obtain the organic loadings required for this investigation. The flow rates were determined by measuring the influent and effluent volumes at fixed time invervals. Since the synthetic waste was made up with tap water, which has a very small number of bacteria, and since flow rates were high, refrigeration of the carboy was unnecessary.

The operating temperature of the unit was $23^{\circ}C \pm 2^{\circ}C$ and evaporation losses, as determined by measuring influent and effluent volumes, were negligible.

The continuous unit was monitored daily for pH and suspended solids. These measurements indicated required pH adjustment when necessary, and determined the quantity of solids to be wasted in order to keep the organic loading to the unit constant.

## 3-2 Batch Aeration Units

Two similar plexiglass batch units containing five compartments were used. The capacities of the individual compartments were 1.5 and 2.0 liters, respectively. The units were tall and narrow and the inside corners were beveled to provide good mixing. Air was provided by fritted stone diffusers mounted on the wall of the compartments about 1.5 inches from the bottom. Samples were removed via sample ports located 3 inches from the bottom of the units and extending one inch into the interior. Photographs of both types of laboratory units are shown in Figure 3-2.

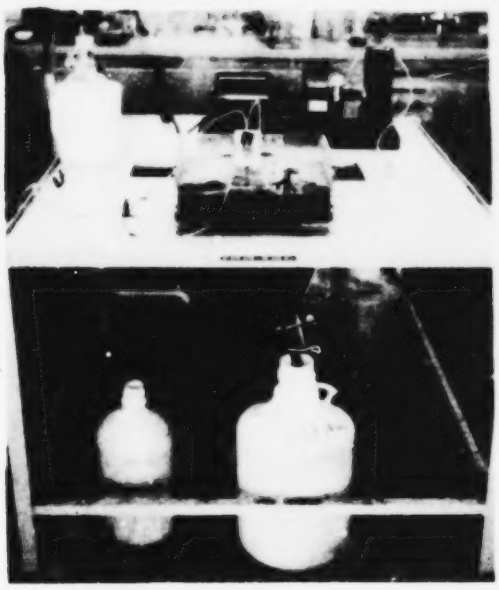

Fig. 3-2 (a)
Continuous Activated Sludge Unit

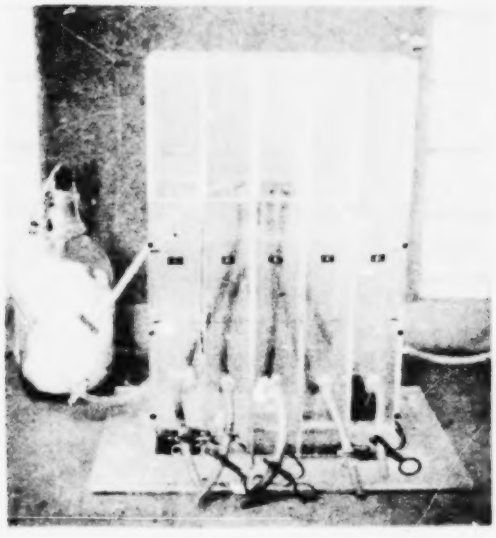

Fig. 3-2 (b)
Batch Activated Sludge Unit

## 3-3 Experimental Procedures

The experiments were designed: (1) to observe the removal of each of the substrates from batch units containing the individual substrates, (2) to follow the removal of the substrates from units in which they were mixed together, (3) to observe the effect of initial substrate concentration on the substrate removal rate, (4) to note the effect of active biological solids on the substrate removal rates, and (5) to measure the rate of organic carbon removal, as measured by COD from the batch units listed above.

The synthetic waste used to acclimate the sludge in the continuous unit was made up daily. A calculated volume of stock solution containing the substrates and inorganic nutrients was diluted to the desired total volume with tap water. Nitrogen and phosphorus were supplied in the BOD : N : P ratio of 100 : 5 : 1 and the pH of the solution was adjusted to 7.5 with sulfuric acid. Table 3-1 gives the composition of the stock solutions used in this investigation.

Activated sludge seed, obtained from the Govalle Sewage Treatment Plant in Austin, Texas, was acclimated to the synthetic waste in a 10-liter continuous-flow aeration unit. The progress of acclimation was followed by measuring effluent COD and the biological solids build-up. Solids were determined and wasted daily in order to obtain the desired organic loading to the system and the pH of the system was checked at the same interval. The loading to the continuous unit determined the type of sludge, i. e., filamentous of flocculent, which would be developed.

TABLE 3-1

Stock Feed Solution

| | |
|---|---|
| Glucose | 19 g/l |
| Phenol | 9.5 g/l |
| Aniline | 9.5 g/l (9.3 ml/l) |
| Ammonium Sulfate | 15.2 g/l |

100 ml of stock solution diluted to 19 l gives the following substrate concentrations:

| | |
|---|---|
| Glucose | 100 mg/l |
| Phenol | 50 mg/l |
| Aniline | 50 mg/l |

Phosphate Buffer Solution

| | |
|---|---|
| $KH_2PO_4$ | 75 g/l |
| $K_2HPO_4$ | 75 g/l |

Bicarbonate Buffer Solution

| | |
|---|---|
| $NaHCO_3$ | 100 g/l |

Periodically samples were taken from the continuous unit and were analyzed for glucose, phenol, aniline, COD, and total carbon in order to compare them with the batch data and determine the extent of their removal in the continuous system.

When the sludge was acclimated to the synthetic waste at the desired organic loading, the sludge was transferred to the batch units for the substrate removal tests. Pre-determined, concentrated samples containing enough substrate and nutrients to give the desired concentrations in the batch units were then added. After allowing approximately two minutes for complete mixing, the first samples were taken. Samples were then taken at selected intervals throughout the run, generally about one hour. The biological solids (MLVSS) in each of the batch units were measured at the beginning and end of each batch run.

The samples removed from the batch units were immediately filtered through Whatman Number 42 filter paper using a 9 cm. Buchner funnel and a vacuum pump adjusted to approximately 10 inches of mercury. This procedure removed essentially all of the biological solids from the sample. It was recognized that this procedure would not remove individual bacteria from solution, but the removal obtained was deemed sufficient for the objectives of this investigation. The samples were then acidified to about pH 1.5 with concentrated sulfuric acid and stored in a refrigerator at 4°C until analysis. All of the analyses were performed within approximately one week from the time of the batch run. Samples on which the biochemical oxygen demand was run were filtered and run immediately after sampling the batch units.

The samples were analyzed for glucose, phenol, aniline, chemical oxygen demand, and in some cases, biochemical oxygen demand and total organic carbon. Total organic carbon was used to show that the chemical oxygen demand as measured by the dichromate method gave an accurate criterion of the carbonaceous material present. Only filtered samples were analyzed since only the soluble substrate was of interest.

# Chapter 4

# LABORATORY ANALYSES

Approximately 100 ml of filtered batch or continuous sample was required for the analyses performed. The samples were analyzed for chemical oxygen demand, glucose, aniline, and phenol concentrations and in some cases, for total organic carbon and biochemical oxygen demand. Mixed liquor total (MLSS) and volatile suspended solids (MLVSS) were also measured in both the batch and continuous units.

## 4-1 Solids Determinations

Both total and volatile mixed liquor suspended solids were measured in the batch and continuous units using the gooch crucible method with glass fiber filters as prescribed in Standard Methods (2). The continuous unit was sampled by first plugging the effluent wier and then raising the baffle to give a completely mixed system before withdrawing the sample. This determination was used to compute the net sludge synthesized and the corresponding volume of solids to be wasted in in order to keep the organic loading to the continuous system constant. In the batch system, the required volume of sample was withdrawn directly at the desired sampling time.

## 4-2 Sample Preparation

The samples were withdrawn from the units into 100-ml beakers at the prescribed sampling times. These samples were then filtered through

Whatman number 42 filter paper in 9-cm Buchner funnels and the filtrate collected in a 500-ml Erlenmeyer flask under ten inches of mercury vacuum. The samples were then acidified to approximately pH 1.5 using concentrated sulfuric acid. The samples were then put in polyethylene bottles and stored in a refrigerator until analysis.

## 4-3 Chemical Oxygen Demand

Chemical oxygen demand (COD), measured by the dichromate reflux method as prescribed in Standard Methods (2), was used as an estimate of the organic content of the filtered samples. COD was considered practical for this purpose since all the compounds used as substrates give essentially one hundred percent of their theoretical chemical oxygen demand.

The COD determination was automated by using the Technicon Auto Analyzer. Since a large number of samples were analyzed, this method greatly reduced the time required. This procedure is discussed in Appendix B-1.

## 4-4 Glucose Determination

Glucose in the filtered samples was determined by using the colorimetric anthrone reaction. Anthrone is specific for the measurement of carbohydrates, and since glucose was the only carbohydrate used in this investigation, the reaction was specific for glucose. The intensity of the blue or green color formed when anthrone and glucose are mixed with concentrated sulfuric acid is proportional to the concentration of glucose in the sample. This method is an adaptation of the method described in Snell and Snell (36) and used by Miholits (31). (See Appendix C-1)

## 4-5 Aniline Determination

Aniline was determined using two methods. When no phenol was present, aniline could be determined by measurement of the optical density of the sample at 280 mμ on the Beckman model DB ultraviolet spectrophotometer. The optical density of the sample at this wavelength was proportional to the aniline concentration in the sample.

The other method used to determine aniline is a colorimetric method as described on page 198, Volume IV, of Snell and Snell (36). (See Appendix C-2) This method could be used to determine aniline in the presence of phenol, although the results were sometimes erratic. (See p. 51)

## 4-6 Phenol Determination

The concentration of phenol was determined by using the direct photometric method as outlined in Standard Methods (2). The preliminary distillation step was omitted since no interfering substances were present in the synthetic waste. The possibility of aniline interfering with the determination was checked using standards containing the two substances in known concentrations. No interference at the concentrations of aniline used in the investigation was found. The samples were diluted with distilled water in order to obtain concentrations in the range covered by this procedure.

## 4-7 Total Organic Carbon

Total organic carbon (TOC) was used as a check to determine if COD was a valid parameter for estimating the organic carbon content of the samples. The Beckman Carbonaceous Analyzer was used for TOC determinations. This system completely oxidizes the organic carbon to $CO_2$ and measures the $CO_2$ on an infrared analyzer. The instrument and method of operation are described in Appendix B-5.

## 4-8 Biochemical Oxygen Demand

Biochemical oxygen demand (BOD) was determined using the dilution technique in accordance with Standard Methods (2). The BOD test was used to determine the concentration of biodegradable material remaining in the batch units after long aeration periods. All BOD dilutions were run in duplicate to give more precise results.

# Chapter 5

## EXPERIMENTAL RESULTS

All of the activated sludge used in the batch experiments which follow was grown in the continuous unit described previously. The operating data for the continuous unit is presented in Appendix A, since it is not directly applicable to the batch runs. The sludge was acclimated to the concentrations of the substrates which were to be used in the batch tests. Acclimation was normally accomplished in two to three weeks. Organic loading, pH, temperature, and nutrients were kept constant during acclimation of the sludge for each batch run. The activated sludge was transferred directly to the batch units when a run was to be made. This caused a carryover of COD into the batch units equal in concentration to the effluent COD of the completely-mixed continuous unit. However, it was felt that this carryover was preferable to possible changes in the sludge which might occur if it had been washed.

The COD in the effluent from the continuous unit ranged from 30-60 mg/l. The 24-hour samples from the mixed batch units ranged from 50-100 mg/l of COD. The batch units containing one substrate had effluents ranging from 40-80 mg/l of COD. The $BOD_5$ of the filtered effluents from the one substrate units ranged from 2 to 8 mg/l and the total $BOD_5$ for the mixture of the three compounds was from 10-15 mg/l.

It is believed that this residual or carryover organic material could have come from two sources. Since millipore filters were not used to filter the samples, individual bacteria were probably present in the filtered samples. These bacteria could have constituted part of the residual

COD. However, the residual COD tended to rise with increasing substrate concentration, regardless of the biological solids concentration; therefore indicating that possibly bacterial by-products or non-biodegradable organic contaminants in the feed were responsible for the residual COD. Generally, none of the organic compounds fed to the unit could be found in the effluent in significant concentrations.

## 5-1 General

The three simple organic compounds used in this investigation were: the hexose, glucose; the aromatic amine, aniline; and phenol. The aldehyde butanal was used instead of aniline for the first batch experiment. This run indicated that the butanal was rapidly air-stripped from solution, so it was replaced by aniline for the remainder of the investigation.

Two techniques were used to measure the removal of the organic compounds. Each of the compounds was measured by an analytical technique which measured the compound specifically. This made it possible to observe the removals of each of the compounds individually when a mixture of the organic compounds was tested. Chemical oxygen demand was also used in order to give an overall estimate of organic content and as a comparison with the other methods of analysis.

It was found that under the conditions of the investigation, all three compounds were removed linearly, as is shown in Figure 5-1. (The cutoff concentrations at which linear removal ceased are given in Table 6-1.)

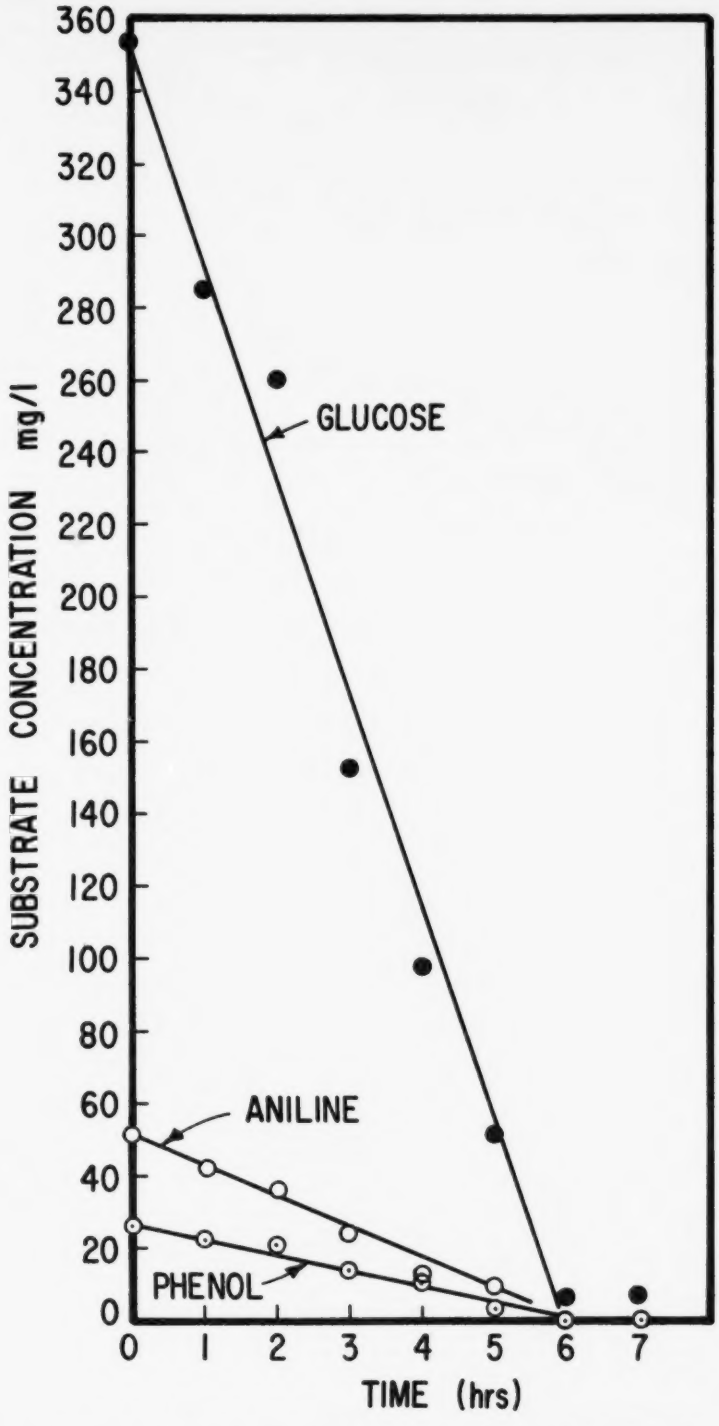


FIG. 5-1. BATCH REMOVAL OF SPECIFIED COMPOUNDS

Figure 5-2 demonstrates that substrate removal as measured by COD was also zero order. Total organic carbon was used as an additional substrate parameter during the last three batch runs. Figure 5-3 indicates that substrate removal measured as TOC removal was also linear. The peculiar lag noted for phenol in this figure will be discussed later. The TOC concentrations in Figure 5-3 have been corrected for the carryover TOC from the mixed unit.

The analytical techniques by which each substrate was specifically measured were used to follow the removal of each compound when a mixture of the three was added to the sludge. Two mixed units were used for most of the batch runs. One contained the substrates in the concentrations used in the continuous unit and the individual batch units, while the other used randomly selected concentrations of each of the substrates. In Figure 5-4 it can be seen that the removal rates of the individual compounds remained unchanged in the presence of the other compounds. In general, this independent removal occurred throughout the batch studies. In several instances, which will be discussed in more detail in a later section of this report, there appeared to be minor effects on the removal of one compound by the presence of another.

The effect of the organic loading to the continuous unit on the activated sludge characteristics was also studied. Many investigators have noted the effect of this parameter on the morphological characteristics of the sludge. Microscopic examinations of the sludges at the various loadings used in this investigation agreed with these previous studies. Photomicrographs of activated sludge at several loadings are shown in Figure 5-5.

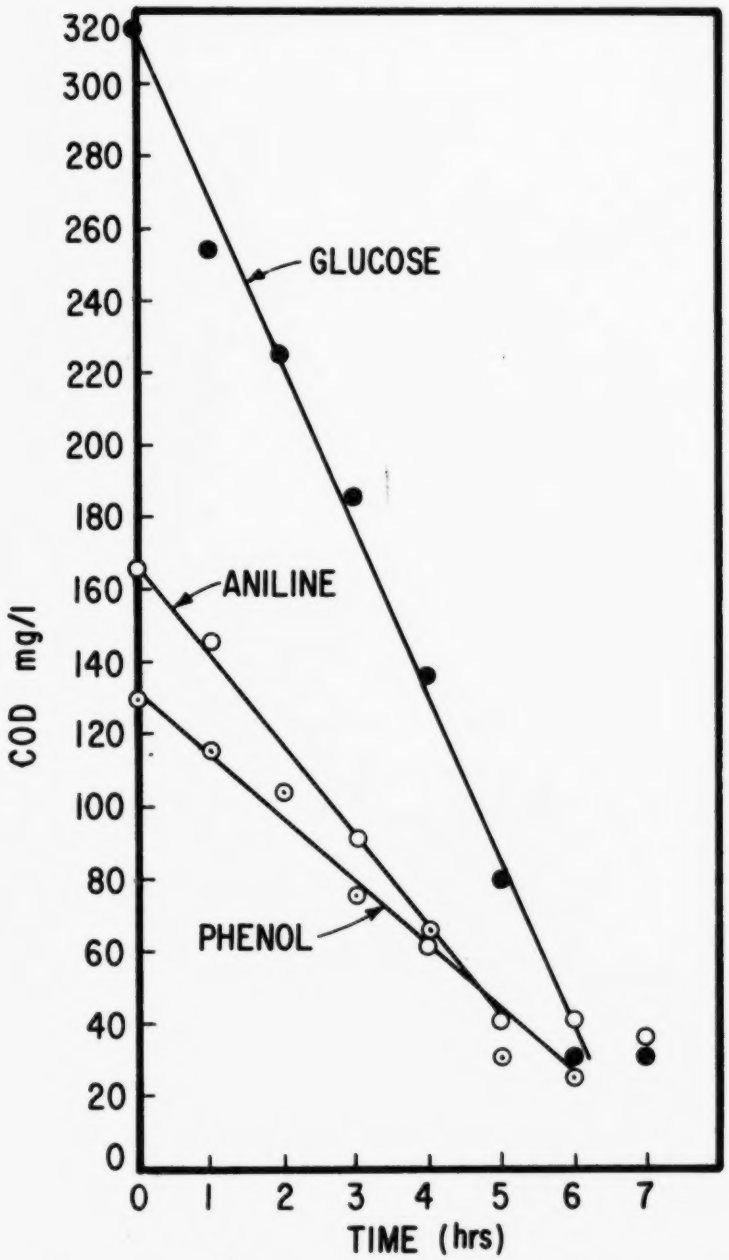


FIG. 5-2. BATCH REMOVAL OF SPECIFIED COMPOUND AS MEASURED BY COD

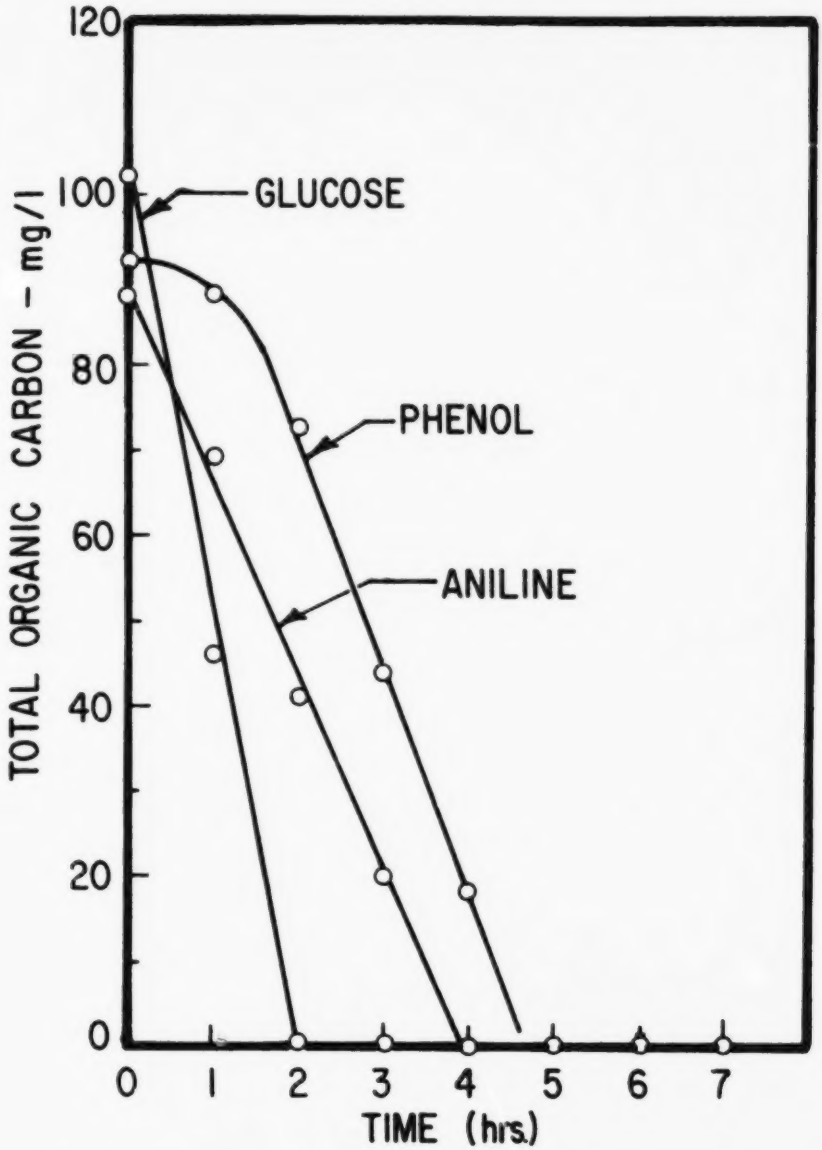


FIG. 5-3. BATCH REMOVAL OF SPECIFIED COMPOUND AS MEASURED BY TOC

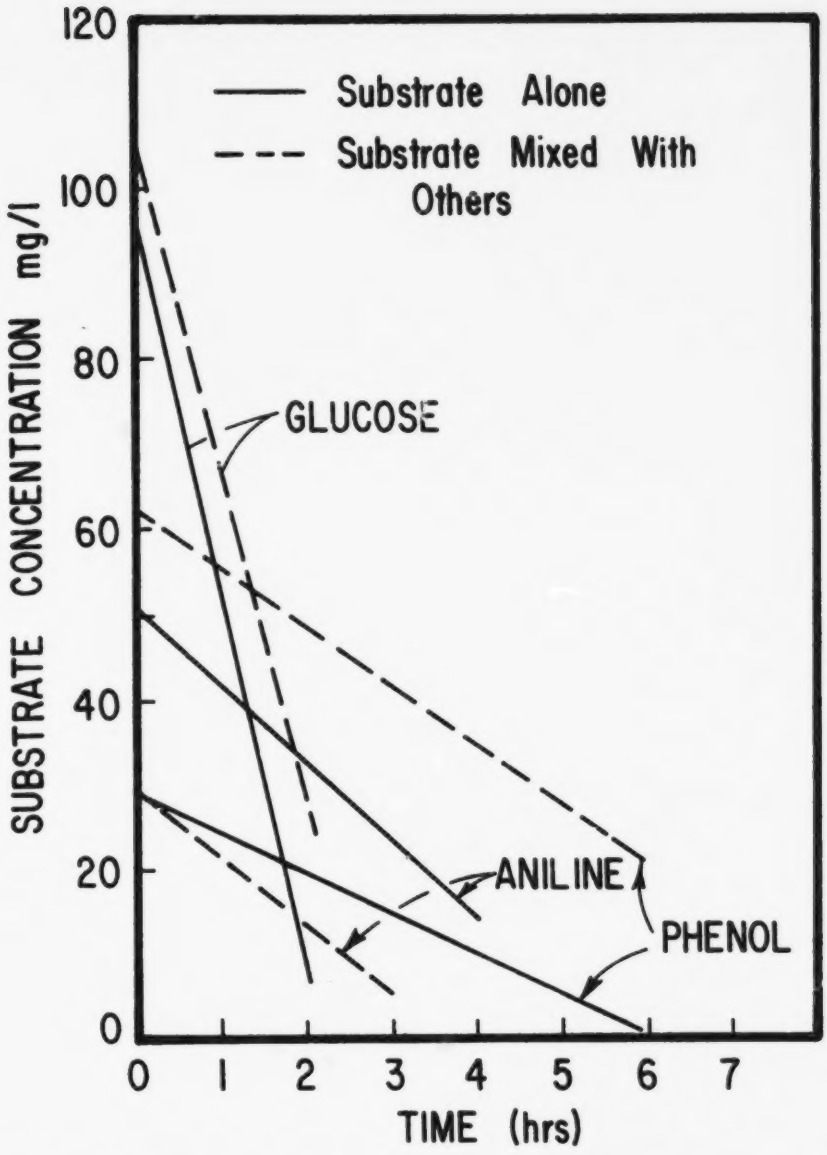


FIG.5-4. BATCH REMOVAL WITH SUBSTRATE ALONE COMPARED TO SUBSTRATE REMOVAL FROM A MIXTURE OF ALL SUBSTRATES

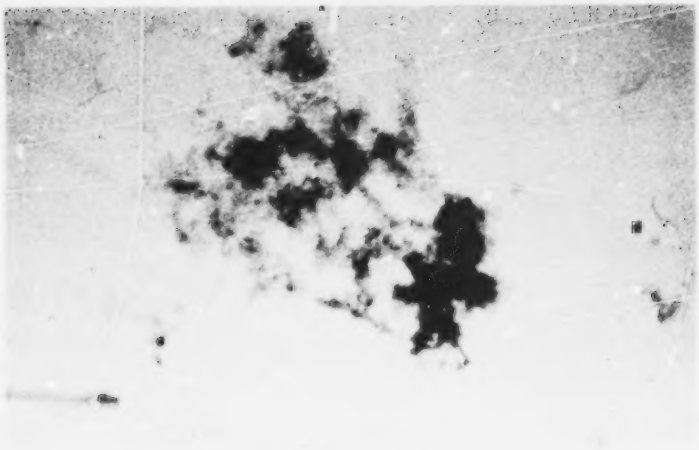

Figure 5-5 (a)
$L_f'=0.2$

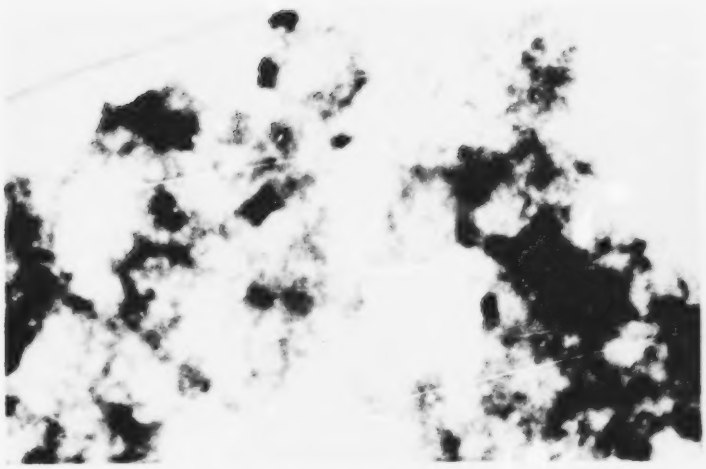

Figure 5-5 (b)
$L_f'=0.45$

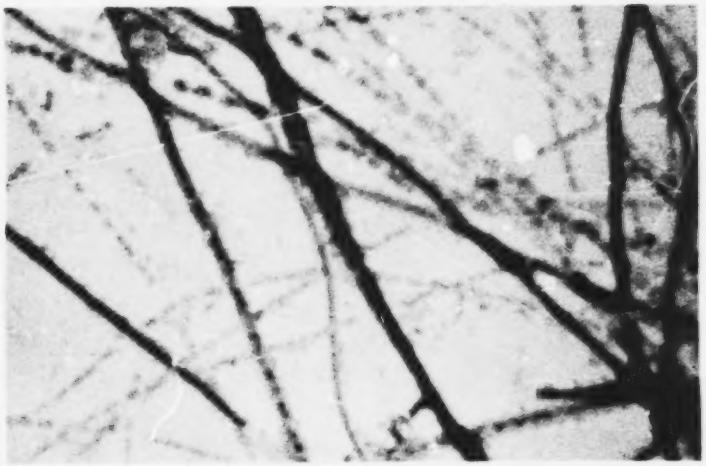

Figure 5-5 (c)
$L_f'=1.2$

These photos are representative of the sludge at similar loading levels in all the experiments.

Figure 5-5 (a) is typical of a sludge at a low loading level. It is a flocculent sludge, with some dispersed particles. A highly flocculated sludge characteristic of intermediate organic loadings is shown in Figure 5-5 (b). Figure 5-5 (c) represents a highly filamentous sludge which was found at high loadings. This sludge was a typical "bulky" sludge as described in the literature No attempt was made in this investigation to identify the microorganisms as to genus and species.

The primary purpose for varying the organic loading to the continuous unit was to observe the effect on the removal rates of the simple compounds in the batch tests. The data indicated that the filamentous sludge gave greater removal rates for all three compounds, as demonstrated in Figure 5-6. These rates were nearly always higher, sometimes by an order of magnitude, than the rates obtained with the flocculent sludges.

## 5-2 Glucose Removal

The simple hexose sugar, glucose, was the most readily assimilable substrate used in this investigation. The glucose removal rates were normally unaffected by the presence of the other two compounds, as indicated in Figure 5-4. However, phenol concentrations above 150 mg/l tended to retard the glucose removal rates in the mixed unit. The data in Figure 5-7 for the batch runs of June 19 show this effect. This change in removal rate appeared to be due to an inhibitory effect on the glucose-utilizing organisms rather than a competitive effect by the phenol and occurred only

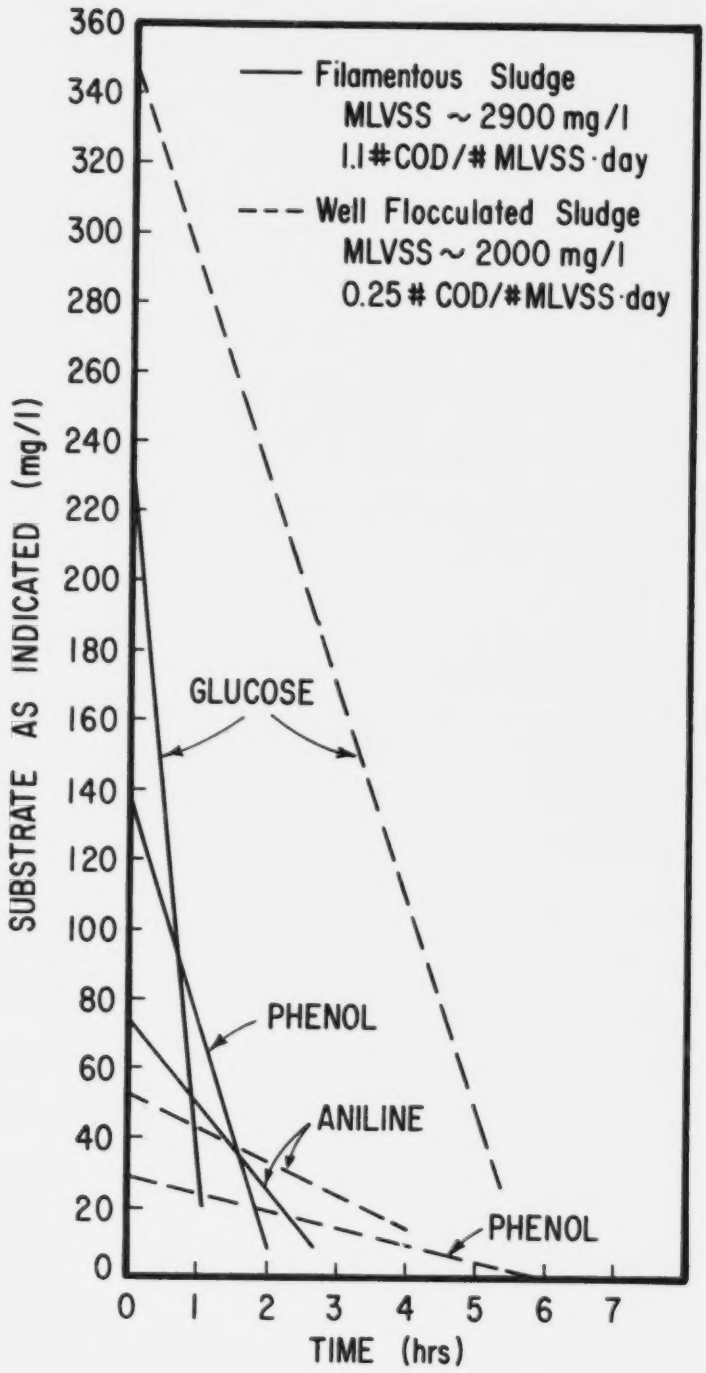


FIG. 5-6. EFFECT OF SLUDGE CHARACTERISTICS ON SUBSTRATE REMOVAL RATES

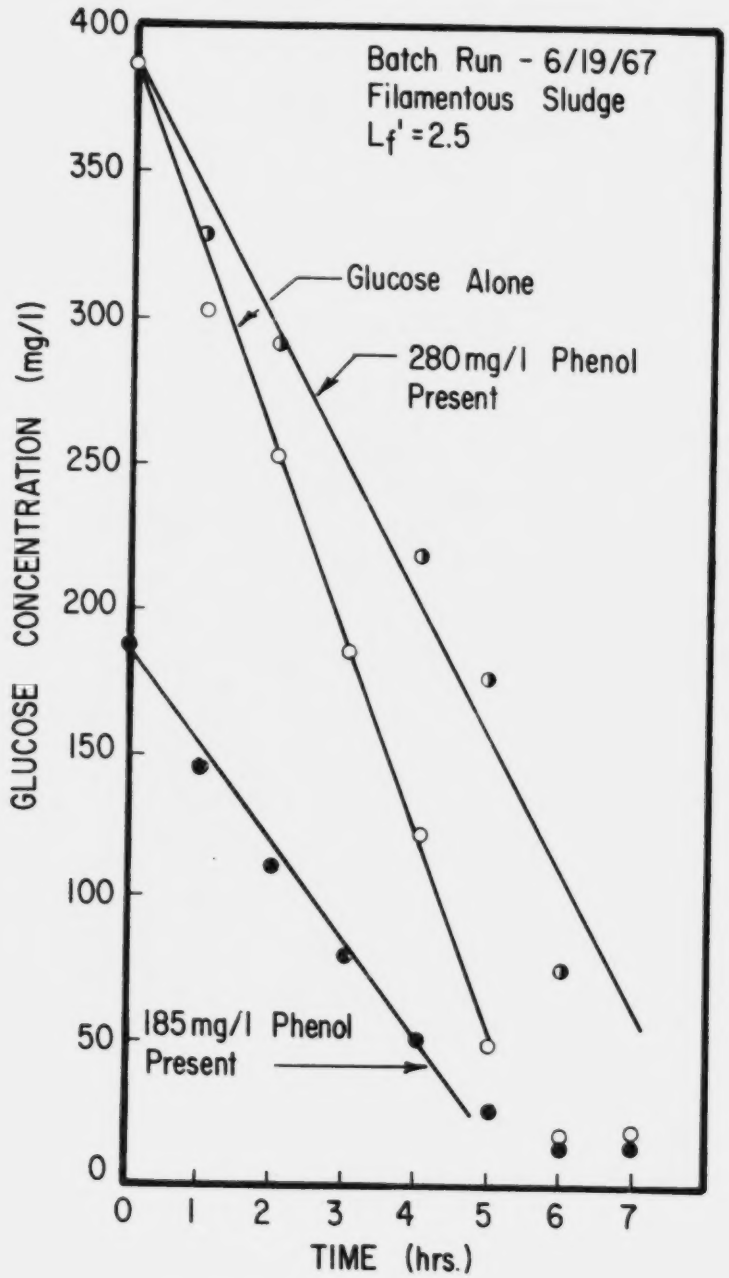


FIG. 5-7. EFFECT OF HIGH PHENOL CONCENTRATIONS ON GLUCOSE REMOVAL

at these high concentrations. All of the glucose removal data is summarized in Table 5-1.

Effect of Glucose Concentration

The initial concentration of glucose added to the batch and continuous units was changed to observe the effect of this variable on the glucose removal rate. Figure 5-8 shows the data obtained for both non-filamentous and filamentous sludges. The data for the non-filamentous sludges indicates that the removal rate per unit of biological solids did not vary with substrate concentration. The filamentous data, although high scattered, also shows no definitive effect of glucose concentration on the removal rate.

Effect of Organic Loading

The effect of organic loading on the morphology of the activated sludge was previously shown. The data in Figures 5-8 and 5-9 indicate that the filamentous sludges gave higher glucose removal rates than the flocculent sludges. Considering the two types of sludge separately, loading had no effect on the assimilative capacities of the sludge. This data is summarized in Table 5-1.

Effect of Biological Solids

Figure 5-9 demonstrates the effect of biological solids (MLVSS) on the glucose removal rates. The data, although scattered, indicates that the rate of glucose removal increased with increasing microorganism

Table 5-1

GLUCOSE REMOVAL DATA

| Run No. | Loading (COD Basis) | Glucose (mg/l) | MLVSS (mg/l) | Glucose Removal Rates mg/l/hr | Unit Solids $hr^{-1}$ | COD Removal Rate $hr^{-1}$ |
|---|---|---|---|---|---|---|
| | | | Filamentous Sludge | | | |
| 618A | 2.5 | 386 | 970 | 65 | 0.067 | 0.09 |
| 618D* | 2.5 | 386 | 860 | 49 | 0.057 | |
| 618E* | 2.5 | 186 | 880 | 35 | 0.04 | |
| 722C | 1.2 | 200 | 1980 | 185 | 0.077 | 0.046 |
| 722D* | 1.2 | 195 | 1900 | 153 | 0.097 | |
| 1020C | 1.1 | 240 | 2820 | 202 | 0.071 | 0.074 |
| 1020D* | 1.1 | 280 | 2870 | 220 | 0.077 | |
| 1020G | 1.1 | 120 | 2930 | 72 | 0.024 | 0.019 |
| 518A | 1.1 | 280 | 1941 | 127 | 0.065 | 0.075 |
| 518D* | 1.1 | 268 | 2110 | 101 | 0.048 | |
| 518E* | 1.1 | 184 | 1985 | 90 | 0.046 | |
| | | | Non-Filamentous Sludge | | | |
| 425A | 0.52 | 174 | 2330 | 85 | 0.036 | 0.049 |
| 425D* | 0.52 | 174 | 2480 | 67 | 0.027 | |
| 425E* | 0.52 | 296 | 2460 | 88 | 0.036 | |
| 46A | 0.42 | 316 | 3700 | 132 | 0.036 | |
| 46D* | 0.42 | 324 | 3720 | 109 | 0.029 | |
| 46E* | 0.42 | 100 | 3600 | 80 | 0.022 | |
| 916A | 0.32 | 73 | 2580 | 96 | 0.037 | 0.085 |
| 916D* | 0.32 | 89 | 2460 | 83 | 0.034 | |
| 29A | 0.25 | 97 | 1610 | 43 | 0.027 | 0.050 |
| 29D* | 0.25 | 104 | 1490 | 38 | 0.025 | |
| 29E | 0.25 | 352 | 1650 | 60 | 0.036 | 0.029 |
| 39A | 0.2 | 100 | 2660 | 85 | 0.032 | 0.030 |
| 39D* | 0.2 | 102 | 2470 | 70 | 0.028 | |
| 39E* | 0.2 | 50 | 2750 | 80 | 0.029 | |
| Grown on 5 Substrates: | | | | | | |
| 627A | 0.65 | 172 | 2400 | 68 | 0.028 | 0.034 |
| 627D* | 0.65 | 136 | 2420 | 40 | 0.017 | |

* Units with mixture of substrates

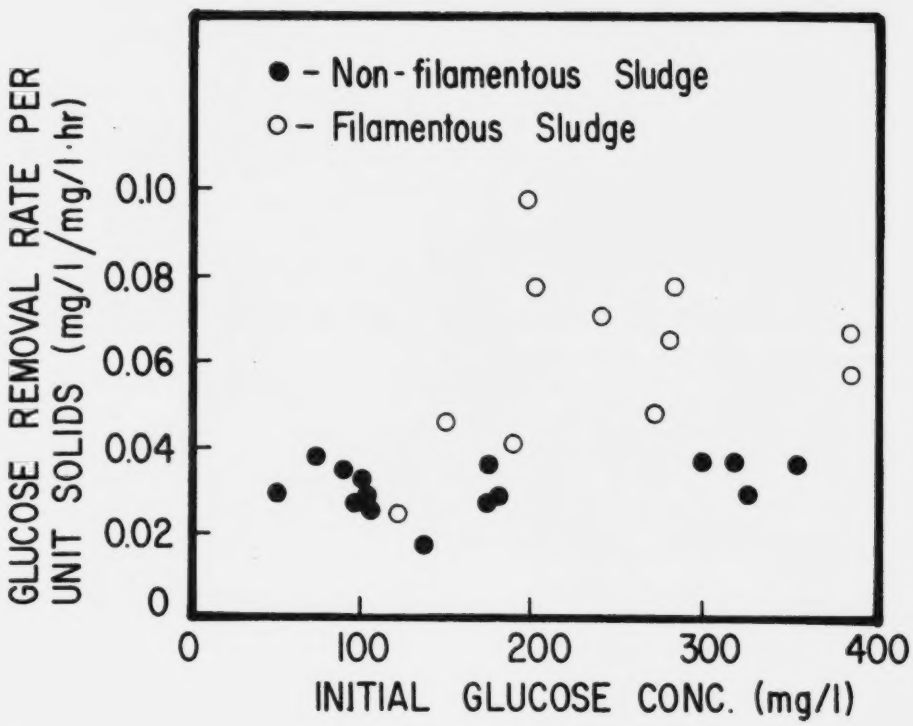


FIG. 5-8. EFFECT OF GLUCOSE CONCENTRATION ON REMOVAL RATE

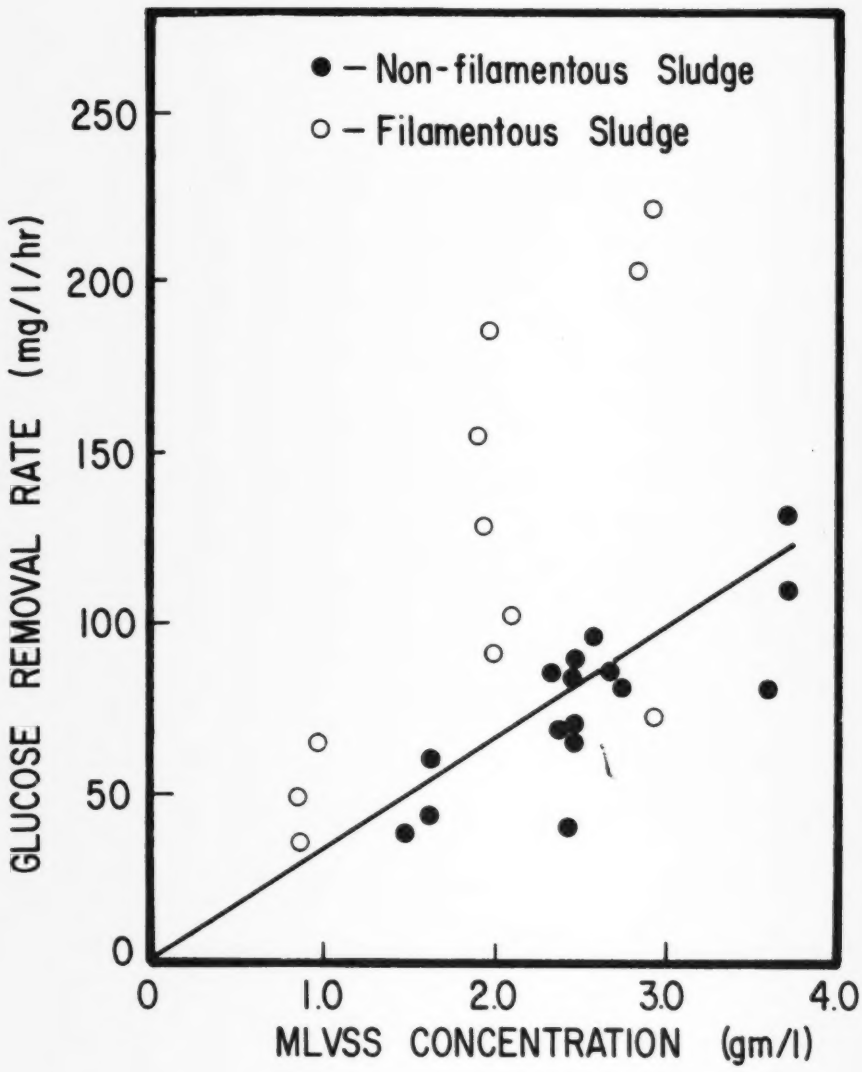


FIG. 5-9. EFFECT OF BIOLOGICAL SOLIDS ON GLUCOSE REMOVAL RATE

concentration. The filamentous sludges gave a greater rate increase for a given increase of solids than the flocculent sludge, as shown in the figure. This finding indicates that the filamentous sludges had a higher assimilative capacity for glucose than the flocculent sludges. This higher removal probably occurs because the filaments have a greater surface area per unit weight than the flocculent organisms. Since filamentous sludges are often found in practice when wastes with a high carbohydrate concentration are treated, this result explains their occurrence.

## 5-3 Phenol Removal

Phenol is often considered as an inhibitory compound for microbial systems. The experiments indicated that phenol could be readily utilized by a properly acclimated activated sludge. The data presented in Figure 5-1 indicate that phenol removal was linear at the concentrations used in this investigation. However, some inhibitory effects were noted at the higher phenol concentrations used.

The TOC data shown in Figure 5-3 indicate an initial lag in phenol removal for this run. This lag occurred frequently in the batch studies and usually lasted about two hours. At the end of the lag period, removal invariably became linear and the phenol was removed to levels below 1 mg/l. This lag in phenol removal was not evident when the phenol was mixed with other substrates. Apparently the inhibitory effects of the phenol were greater when the sludge was exposed to this compound alone. The phenol data in Table 5-2 indicate that phenol removal in the mixed units proceeded at rates equal to the linear removal rates in the batch units with phenol alone.

Table 5-2

PHENOL REMOVAL DATA

| Run No. | Loading (COD Basis) | Phenol mg/l | MLVSS mg/l | Phenol Removal Rates mg/l/hr | Unit Solids $hr^{-1}$ | COD Removal Rate $hr^{-1}$ |
|---|---|---|---|---|---|---|
| | | | Filamentous Sludge | | | |
| 618B | 2.5 | 260 | 860 | 4.5 | 0.005 | |
| 618D* | 2.5 | 280 | 860 | 5 | 0.006 | |
| 618E* | 2.5 | 185 | 880 | 4.5 | 0.005 | |
| 722A | 1.2 | 88 | 1980 | 28 | 0.014 | 0.036 |
| 722D* | 1.2 | 96 | 1900 | 29 | 0.015 | |
| 1020A | 1.1 | 140 | 2990 | 41 | 0.022 | 0.048 |
| 1020D* | 1.1 | 88 | 2870 | 65 | 0.014 | |
| 1020E | 1.1 | 147 | 2950 | 88 | 0.030 | 0.059 |
| 518B | 1.1 | 93 | 1930 | 23.5 | 0.012 | 0.036 |
| 518D* | 1.1 | 178 | 2110 | 31.5 | 0.015 | |
| 518E* | 1.1 | 127 | 1990 | 22 | 0.011 | |
| | | | Non-Filamentous Sludge | | | |
| 425B | 0.52 | 46 | 2380 | 17 | 0.0076 | |
| 425D* | 0.52 | 98 | 2480 | 30 | 0.012 | |
| 425E* | 0.52 | 74 | 2470 | 29.5 | 0.012 | |
| 46B | 0.42 | 75 | 3550 | 15.5 | 0.004 | 0.02 |
| 46D* | 0.42 | 150 | 3720 | 23 | 0.006 | |
| 46E* | 0.42 | 93 | 3600 | 29.5 | 0.008 | |
| 916B | 0.32 | 45 | 2540 | Extended lag - rate increasing | | |
| 916D* | 0.32 | 46 | 2460 | when experiment was terminated | | |
| 29B | 0.25 | 25 | 1560 | 4.5 | 0.003 | 0.011 |
| 29D* | 0.25 | 62 | 1490 | 8.5 | 0.006 | |
| 39B* | 0.2 | 46 | 2600 | 12.5 | 0.005 | |
| 39D* | 0.2 | 52 | 2470 | 12 | 0.005 | |
| 39E* | 0.2 | 23 | 2750 | 11 | 0.004 | |
| Grown on 5 Substrates: | | | | | | |
| 627B | 0.65 | 56 | 2380 | 14.5 | 0.006 | 0.024 |
| 627D* | 0.65 | 71 | 2420 | 10.0 | 0.004 | |

* Units with mixture of substrates

It is believed that the lag in phenol removal in the batch units was the result of the method of sludge acclimation used. Since the activated sludge was acclimated to the substrate mixture in a completely-mixed continuous unit, the sludge was actually exposed to a substrate concentration slightly above the effluent concentration. For phenol, this concentration was usually less than 1 mg/l. In the batch tests, the substrates were added to the units to give a zero-time concentration equal to the concentration of the compounds in the influent to the continuous unit. This concentration ranged from 25-200 mg/l for phenol. Since the sludge had been exposed to concentrations considerably lower than these, the phenol exerted a temporary inhibitory effect on the sludge. The sludge recovered from this inhibition and proceeded to remove phenol linearly after a period of about two hours. When this lag phase was noted, the removal rates were computed from the linear portion of the curves.

Phenol removal was high in all runs with the exception of the sludge tested June 18. This sludge was exposed to an organic loading of 2.5 lb COD/lb MLSS·day, the highest used in this investigation. The influent phenol concentration was 200 mg/l and the effluent was 175 mg/l. The MLVSS in the continuous unit was less than 1000 mg/l. In this run, the phenol had an inhibitory effect on glucose removal from the mixed units. Phenol removal by this sludge was very low, as can be seen from the data in Table 5-2 (run 618). After 24 hours of aeration, the mixed liquor in the two mixed units used in this run turned a dark blue. The filtered samples retained this color, indicating that it was in solution. Acidification of the samples caused the disappearance of the blue color. Since the

other two substrates were essentially gone from solution, it is believed that the color was due to an oxidation state of phenol. Injection of one of these samples into a gas chromatograph gave only one peak, at the same location as the peak for phenol under the conditions used.

The observed rates of phenol removal, not withstanding the lag occurring in some runs, were unaffected by the presence of the other substrates, as is demonstrated in Figure 5-4.

Effect of Phenol Concentration

Initial phenol concentration had no effect on phenol removal rates per unit biological solids, as is shown in Figure 5-10. This data indicates that phenol removal rates were independent of substrate concentration for the flocculent sludges. The filamentous sludge data were too scattered to form any conclusive evidence, but the linear removals obtained with this sludge indicate that the removal rates should be independent of phenol concentration. High initial phenol concentrations did tend to cause a lag in the occurrence of the linear removals, as was previously mentioned.

Effects of Organic Loading

Figures 5-10 and 5-11 indicate that phenol removal rates were greater for the filamentous sludges than for the flocculent sludges. The three low removal rates shown for the filamentous sludge at high concentrations of phenol occurred during the batch runs of June 18, which have been previously discussed. The phenol concentrations used were inhibitory in this batch run.

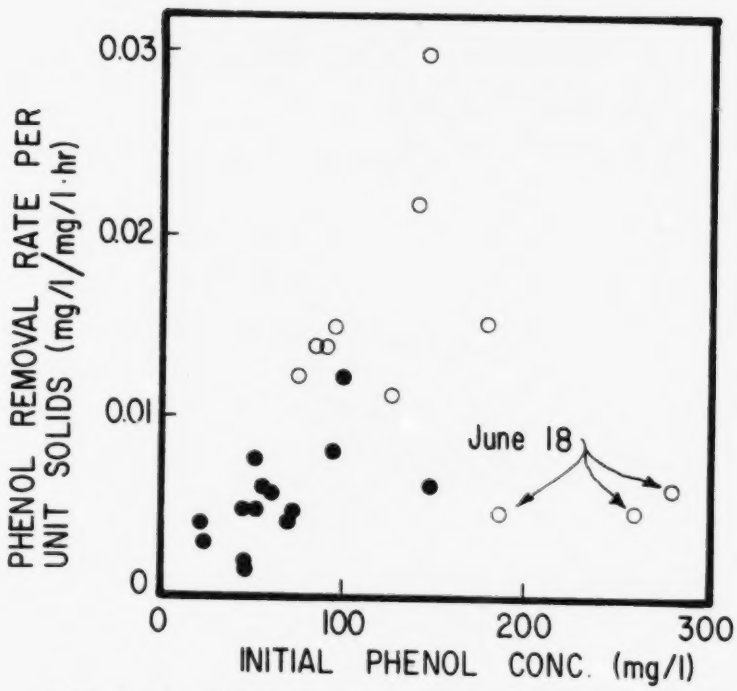


FIG. 5-10. EFFECT OF PHENOL CONCENTRATION ON PHENOL REMOVAL RATE

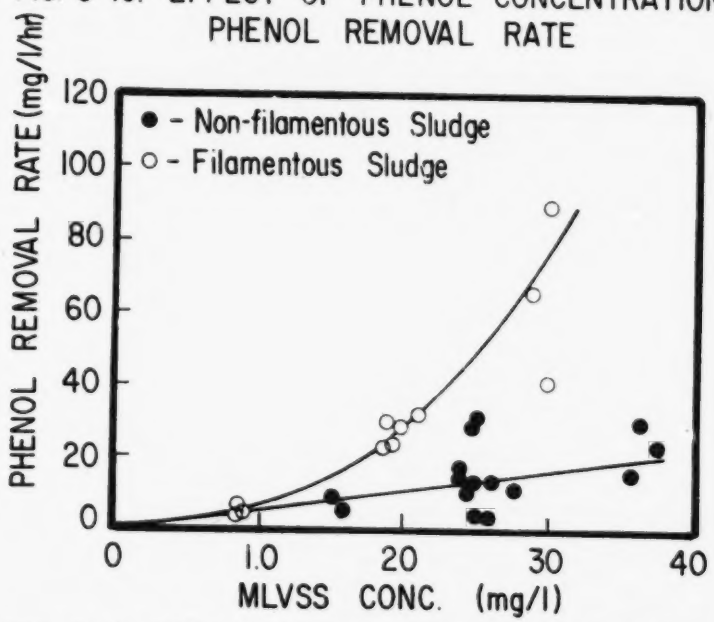


FIG. 5-11. EFFECT OF BIOLOGICAL SOLIDS ON PHENOL REMOVAL RATE

The phenol removal data is summarized in Table 5-2.

Effect of Biological Solids

An increase in biological solids (MLVSS) caused a corresponding increase in phenol removal rate, as shown in Figure 5-11. The filamentous sludges gave a greater increase in phenol removal for a given solids increase than the flocculent sludges did. This observation was similar to findings obtained for glucose. The increase in removal rate was approximately linear for the flocculent sludges, but appeared to be non-linear for the filaments.

5-4 Aniline Removal

The aromatic amine, aniline, was readily utilized by the biological sludges studied. Aniline removal was zero order in all of the batch runs. No inhibitory effects were observed at the concentrations of aniline used.

The aniline data is less complete than that of glucose and phenol because certain difficulties arose when aniline was measured in the activated sludge system. The technique developed measured aniline specifically when glucose and phenol were present. This method was tested many times using standards and the continuous unit influent which had a known aniline concentration. However, in several of the batch runs, the analytical technique measured no aniline, even at zero time. This observation was recorded in both the unit with aniline alone and the mixed unit, therefore indicating there was no interference resulting from the other two compounds.

COD and TOC data from the units containing aniline alone indicated that an organic was present and was removed linearly. In the units containing aniline alone, it was possible to follow the progress of aniline removal using the ultraviolet spectrophotometric techniques. However, when phenol was present, this method could not be used since both compounds absorb ultraviolet radiation at similar wavelengths and the optical densities were not found to be additive.

It is postulated that this inability to measure aniline by the specific method was caused by a rapid change, possibly chemical, of the $NH_2$ group on the aromatic ring, which is the point of reaction for the colorimetric method. The aromatic ring was apparently unchanged since the compound in solution could be measured in the ultraviolet range.

It was found that the rate of aniline removal by the activated sludge was unaffected by the presence of the other organic compounds, as shown in Figure 5-4. The aniline removal data is summarized in Table 5-3.

Effect of Aniline Concentration

The data presented in Figure 5-12 indicate that the aniline removal rates per unit biological solids were independent of aniline concentration. However, the data available for the filamentous sludge were insufficient to lead to any conclusions regarding the effects of substrate concentration on the removal rate. The data for the flocculent sludges conform to the results of the glucose and phenol studies.

Table 5-3

ANILINE REMOVAL DATA

| Run No. | Loading (COD Basis) | Aniline (mg/l) | MLVSS (mg/l) | Aniline Removal Rates (mg/l/hr) | Unit Solids (mg/l /mg/l·hr) | COD Removal Rates (mg/l /mg/l·hr) |
|---|---|---|---|---|---|---|
| | | | Filamentous Sludge | | | |
| 618C | 2.5 | 184 | 848 | 22 | 0.026 | 0.059 |
| 1020B | 1.1 | 156 | 2460 | 23 | 0.008 | 0.01 |
| 1020D* | 1.1 | 168 | 2960 | 36 | 0.012 | |
| 1020F | 1.1 | 72 | 2870 | 24 | 0.008 | 0.017 |
| 518C | 1.1 | 150 | 1985 | - | - | 0.038 |
| | | | Non-Filamentous Sludge | | | |
| 425C | 0.52 | 100 | 2300 | 46 | 0.02 | 0.041 |
| 46C | 0.42 | 150 | 3600 | - | - | 0.025 |
| 916C | 0.32 | 36 | 2540 | 18 | 0.007 | 0.03 |
| 916D* | 0.32 | 39 | 2460 | 20 | 0.008 | |
| 29C | 0.25 | 51 | 1380 | 18 | 0.006 | 0.018 |
| 29D* | 0.25 | 29 | 1490 | 15 | 0.005 | |
| 29G | 0.25 | 150 | 1530 | 18 | 0.006 | 0.018 |
| 39B* | 0.2 | 53 | 2600 | 29 | 0.006 | |
| 39C | 0.2 | 50 | 2700 | 31 | 0.006 | 0.014 |
| 39D* | 0.2 | 51 | 2470 | 27 | 0.006 | |
| 39E* | 0.2 | 53 | 2750 | 31 | 0.006 | |
| Grown on 5 Substrates: | | | | | | |
| 627C | 0.65 | 50 | 2300 | 23 | 0.01 | 0.022 |

* Units with mixture of substrates

Effect of Organic Loading

The data presented in Figures 5-12 and 5-13 indicate that the aniline removal by filamentous sludge was not significantly greater than that by flocculent sludge. This observation is contradictory to findings obtained with glucose and phenol. However, more data regarding the removal rates by filamentous sludge are necessary to verify this finding.

Effect of Biological Solids

The rate of aniline removal increased with increasing biological solids (MLVSS) concentration for the non-filamentous sludges, as illustrated in Figure 5-13. The effect of the MLVSS concentration on aniline removal by the filamentous sludges was not sufficient to permit conclusive comments.

5-5 Removal of Five Simple Compounds

Two additional compounds were added to the three simple organics previously tested in order to observe the effects of these compounds on the removal of the original three components and to observe the COD and TOC removals from a mixture of the five compounds. The two compounds used were the amino acid, glycine, and the organic acid salt, sodium acetate.

Only one set of batch experiments was run with the five component substrate. The acclimation of the activated sludge was performed by the previous method using all five substrates. The organic loading used was 0.65 lb COD/lb Solids/day, which gave a flocculent, good-settling sludge.

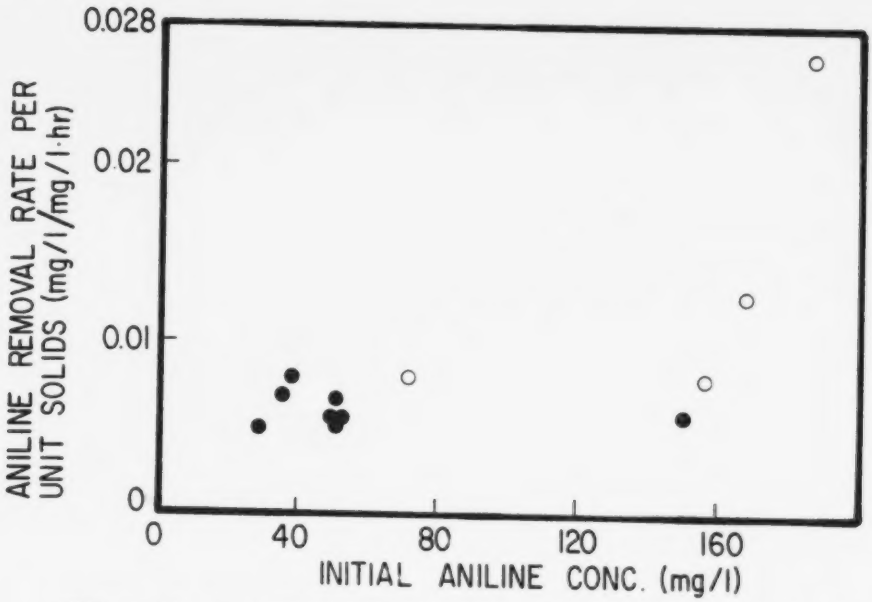


FIG. 5-12. EFFECT OF ANILINE CONCENTRATION ON ANILINE REMOVAL RATE

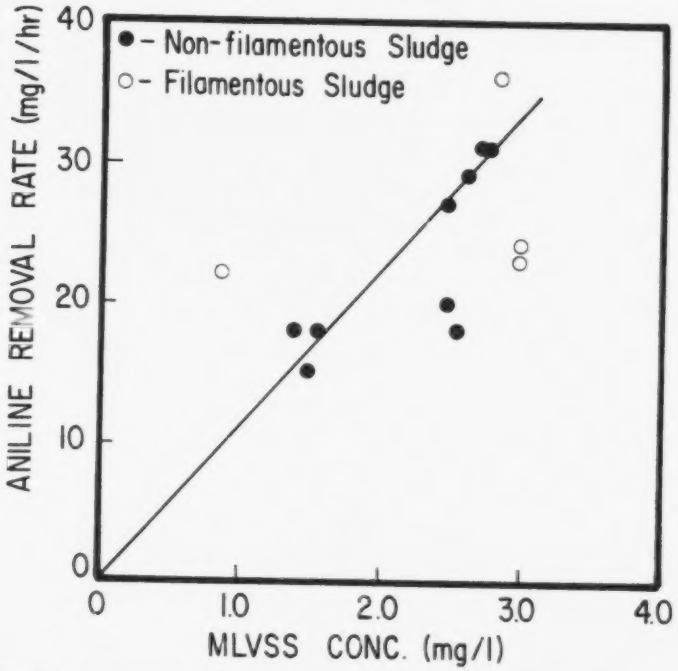


FIG. 5-13. EFFECT OF BIOLOGICAL SOLIDS ON ANILINE REMOVAL RATE

Six batch units were used, one for each of the five substrates alone and one containing a mixture of all five compounds. The concentrations used in the batch tests were those used in the influent to the continuous unit.

Glycine and Acetate Removal

Glycine and acetate concentrations were measured by COD and TOC. Since no specific method was used for the estimation of these two compounds, their removal could only be followed in the batch units containing these compounds alone. Figure 5-14 shows that glycine was removed linearly. The concentration of sodium acetate used was removed too rapidly to give a reliable curve, but the results of studies by Downing (11) indicate that the acetate was probably removed linearly at the concentration used (200 mg/l as sodium acetate). The data in Figure 5-14 were corrected for the carryover TOC concentration from the continuous unit, about 16 mg/l.

Removal of Glucose, Phenol, and Aniline

The removal rates of the three compounds previously tested were unaffected by the addition of glycine and acetate. The summary data in Tables 5-1, 5-2, and 5-3 indicate that the glucose, phenol, and aniline removal rates fall in the range expected for a flocculent sludge, as used in this experiment.

5-6 Mixture Removal

One of the primary objectives of this investigation was to demonstrate that the removal of the individual organic compounds from a mixture,

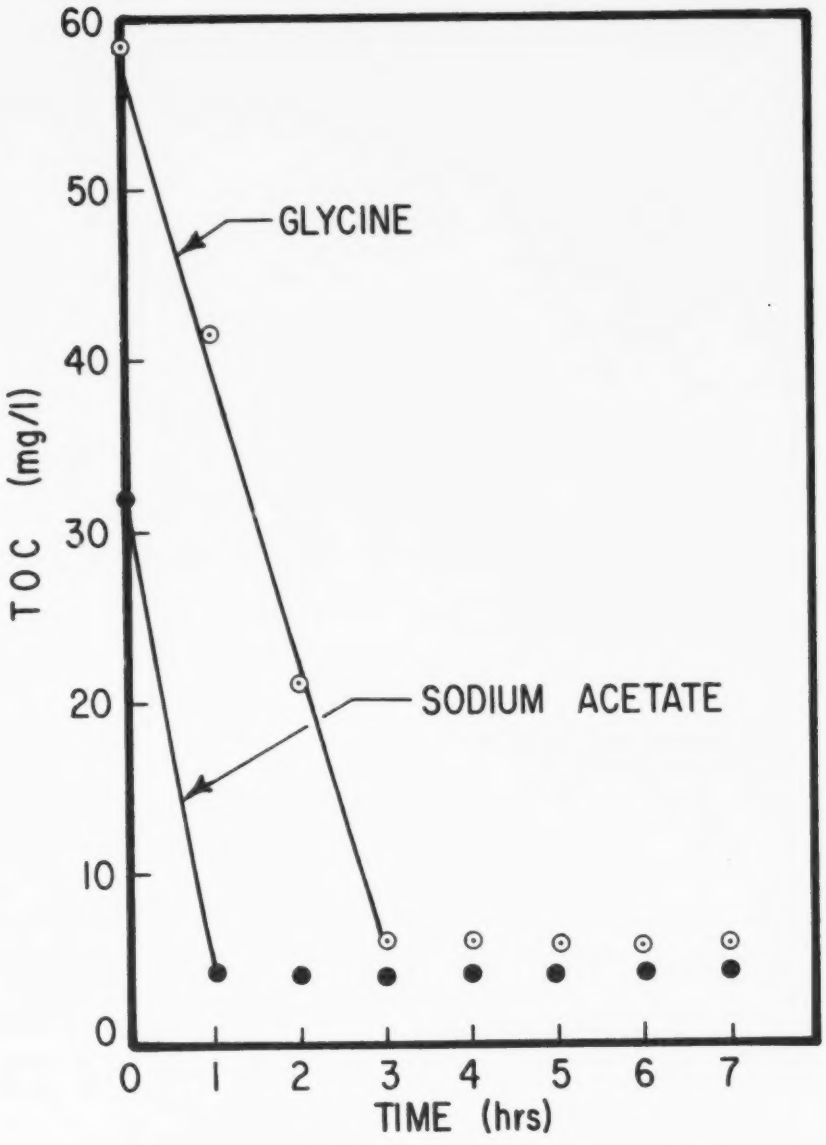


FIG. 5-14. BATCH REMOVAL OF GLYCINE AND ACETATE AS MEASURED BY TOTAL ORGANIC CARBON

as measured by an overall parameter such as COD or TOC, was actually a summation of the linear removals of all its components. In order to demonstrate that COD (or TOC) removals in a mixed waste are the summation of the COD (or TOC) removals of the components of the mixture, the COD's (TOC's) of batch units, each containing one organic compound, were added and compared to the COD (TOC) in a unit which contained a mixture of the compounds in the same concentrations as in the individual units. This comparison was done for each of the sampling times used during the batch runs.

Before these predicted values of COD (TOC) could be accurate, it was necessary to account for the carryover COD (TOC) from the continuous unit. Since this non-degradable organic fraction existed in all of the batch units, summing the data for three batch units would make the residual COD (TOC) of the predicted data three times higher than the residual in the mixed unit. To correct for this error, the residual COD (TOC) was determined for the sludge used and twice this value was subtracted from the summed data from the individual batch units.

COD removals for the mixed units in which the substrate concentrations were randomly selected were predicted by using the theoretical COD computed from the specific substrate removals measured in these units. It was unnecessary to correct for residual COD in the calculations for these units.

COD Removal

Figures 5-15 and 5-16 show that the summed COD data gave a good approximation of COD removal from the mixed unit. In Figure 5-15 a first

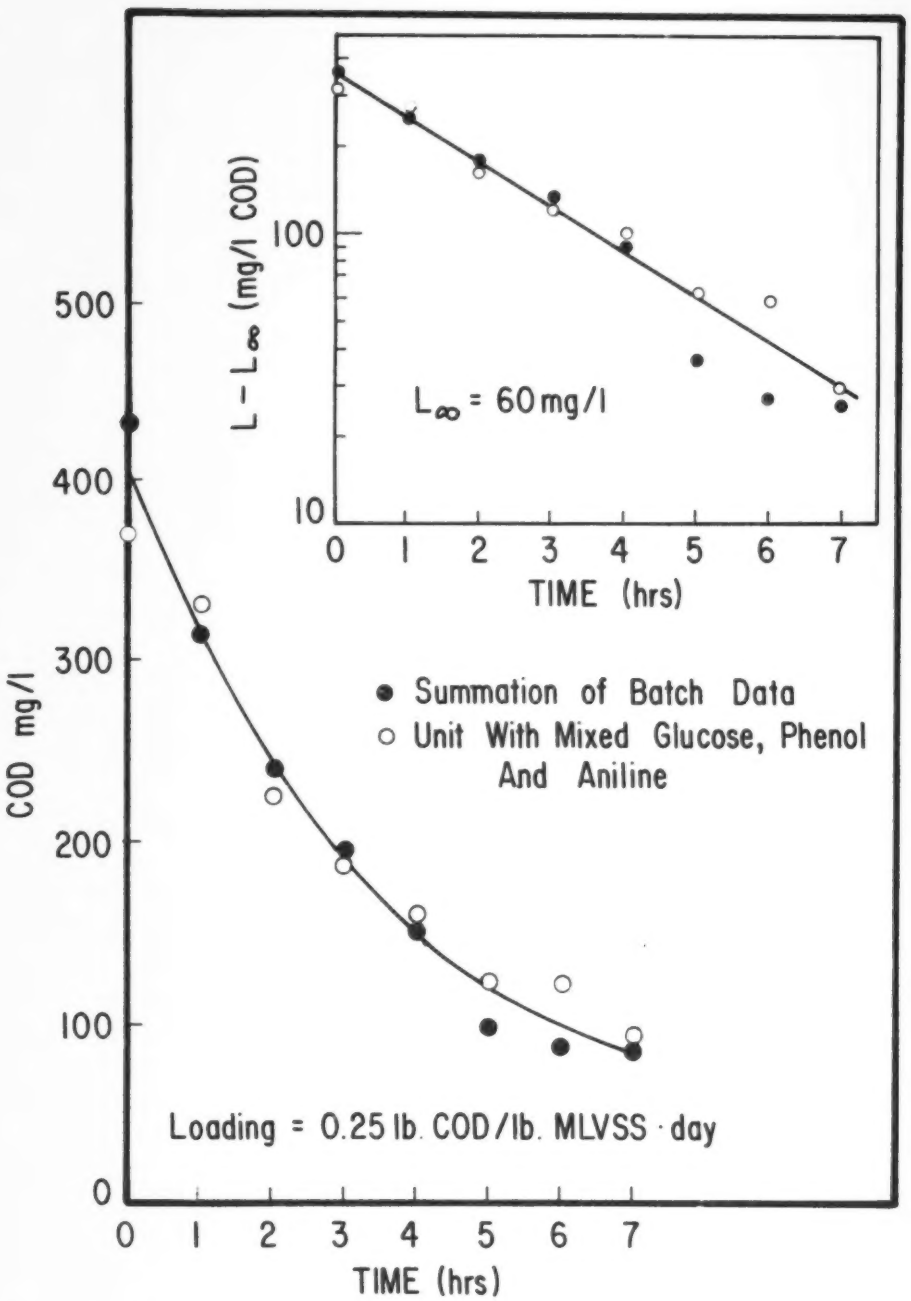


FIG. 5-15. COD REMOVAL COMPARISON - COMBINED COD's FROM SEPERATE UNITS AND COD OF MIXED UNIT WITH SAME INITIAL CONCENTRATION

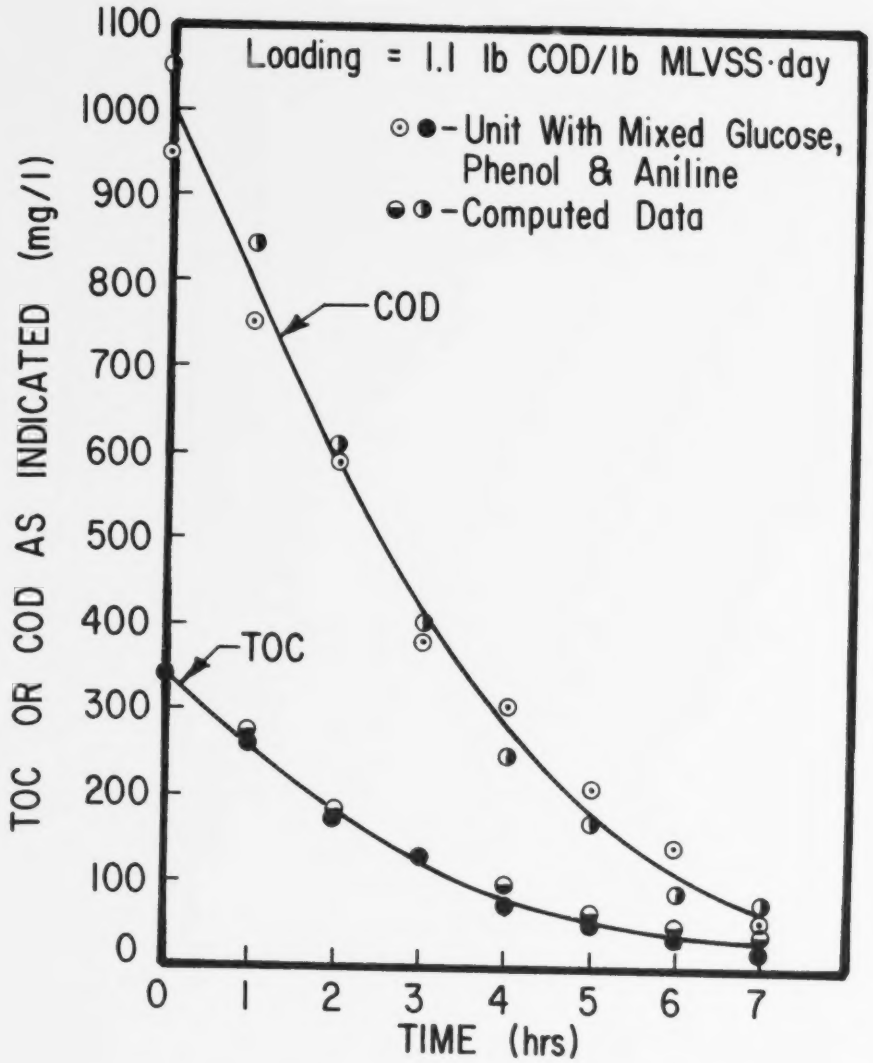


FIG. 5-16. COMPARISON OF COD AND TOC FROM MIXED UNIT WITH VALUES COMPUTED FROM SEPERATE UNIT DATA

order plot of the predicted and measured data is shown. The predicted and measured COD for the other batch runs have been plotted and show identical results. (Appendix A)

TOC Removal

Total organic carbon was used as an overall organic parameter during the last three experiments. Predicted and measured values of TOC compared very well, as demonstrated in Figure 5-16. TOC data tended to be less erratic than COD, probably because the TOC method is more reliable and has fewer interferences.

Five Substrates

Figure 5-17 shows the measured and computed COD and TOC values for the mixed unit containing five simple compounds. The computed and measured values compare very well, as they did for the three substrate system. The predicted values of COD and TOC were corrected for the residual effects due to two additional batch units.

Summary

The results show that, under the conditions of this investigation, the COD and TOC removals by activated sludge from a complex waste could be predicted by summing the COD and TOC removals of the simple compounds which form the complex waste.

In Figure 5-18 the predicted values of COD are plotted against the measured COD's for all of the mixed units used in this investigation.

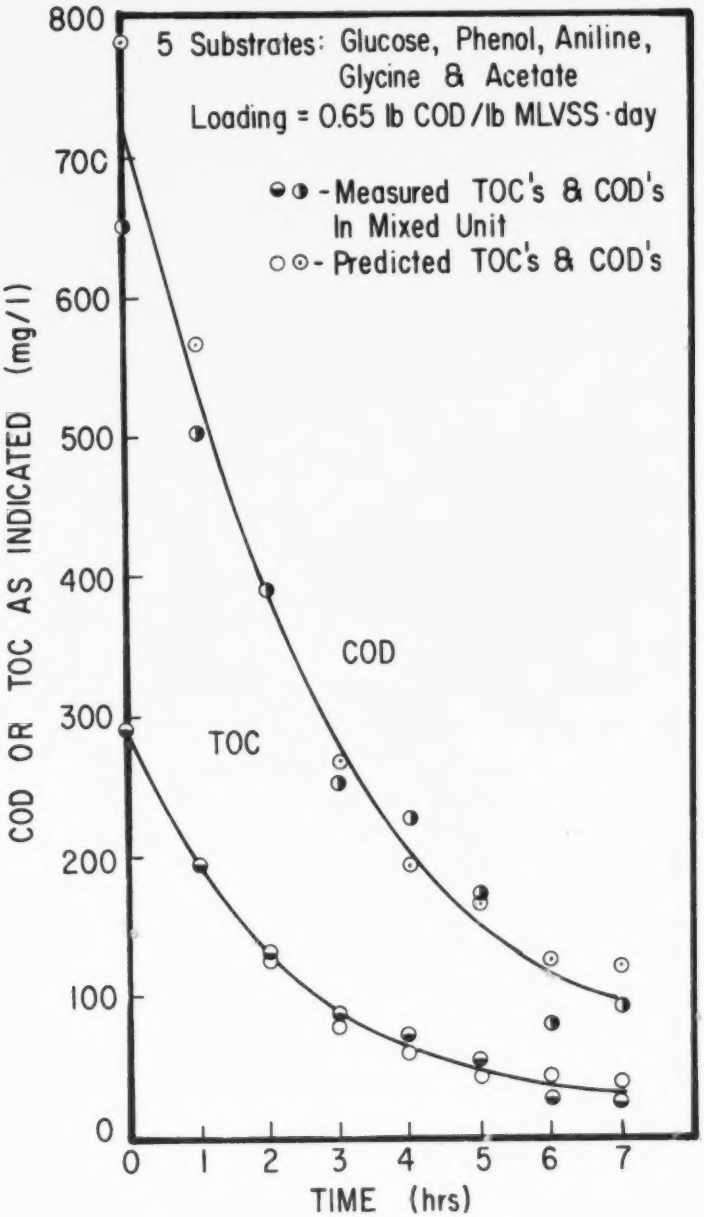


FIG. 5-17. BATCH REMOVAL OF A MIXTURE OF 5 SIMPLE COMPOUNDS AS MEASURED BY COD & TOC

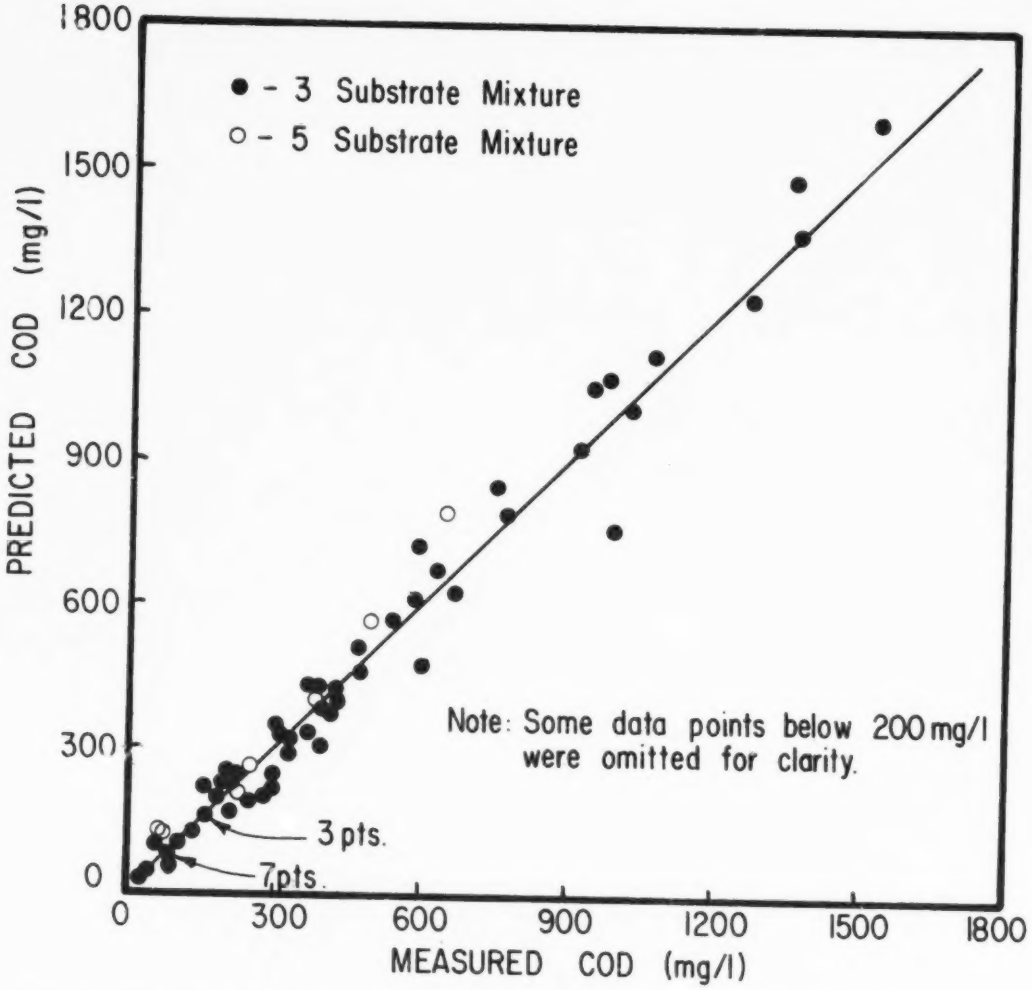


FIG. 5-18. COMPARISON OF MEASURED COD FROM MIXED UNITS WITH VALUES COMPUTED BY SUMMING COD REMOVALS OF SIMPLE COMPOUNDS

This figure indicates a good correlation of computed and measured values.

Figure 5-19 shows the same good approximation, this time using TOC as an overall parameter of organic concentration. TOC seems to give a slightly better prediction of measured values than COD, probably because of the greater precision of the TOC procedure.

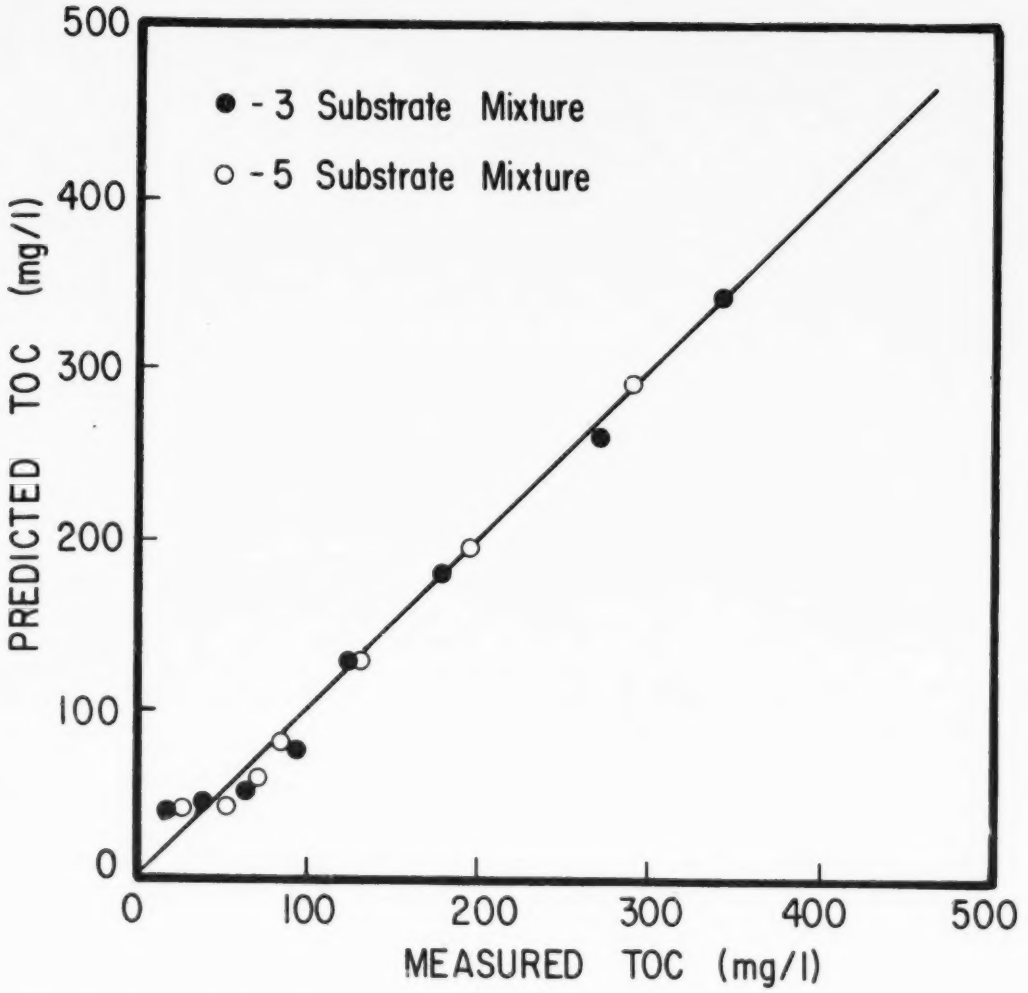


FIG. 5-19. COMPARISON OF TOC'S MEASURED IN MIXED UNITS WITH VALUES COMPUTED BY SUMMING TOC REMOVALS FROM SEPERATE UNITS

# Chapter 6

## DISCUSSION

Batch laboratory tests were used to study the utilization of glucose, phenol, and aniline by an acclimated activated sludge. It was found that all three compounds were removed at rates independent of their concentrations (zero order). According to the Monod equation, the substrate removal rate in the linear removal range should be a constant. Studies in which the initial concentrations of each of the three compounds were varied indicated that this was true for the activated sludge used in this investigation. Linear substrate removal was observed for all of the substrates at the concentrations tested.

The zero order removal rate for a particular compound was unaffected by the presence of the other compounds. Therefore, the sludge was capable of using all three substrates simultaneously, with no preference for a particular compound. This evidence agrees with the data presented by Downing (11) for two simple compounds and tends to support his hypothesis that different organisms are responsible for the removal of each compound. This hypothesis is further substantiated by data observed during studies in which a highly filamentous sludge was almost incapable of using phenol but readily oxidized glucose and aniline. The results of this experiment indicated that most of the phenol-utilizing bacteria had been displaced from the system by the filamentous organisms. However, since it is virtually impossible to determine all the types of microorganisms present in the activated sludge process, this finding is still conjecture.

At high initial phenol concentrations in the batch units, a lag in phenol removal occurred prior to the linear removal of the compound. This effect was inherent in a system in which the sludge was acclimated in a completely-mixed, continuous unit and then fed phenol in a batch system. This procedure gave an instantaneous increase in phenol concentration in the batch unit up to the level present in the influent to the continuous unit. In effect, this rapid concentration increase was analogous to a shock loading of phenol in a continuous process. It seems doubtful that sludge acclimation in a batch unit would eliminate this lag. The linear phenol removals which followed the lags gave rates closely approximating the removal rates in units that had no lag phase. Apparently, the initial lag in phenol removal did not impair the phenol assimilation capacity of the sludge.

High levels of phenol in the mixed batch units exerted an inhibitory effect on glucose removal. Glucose removal was still linear in the mixed unit, but proceeded at a lower rate than in the batch unit with glucose alone. This effect apparently was due to an inhibition of the glucose-utilizing organisms in the activated sludge. Phenol concentrations below 150 mg/l had no effect on glucose removal rates.

Linear removal rates were computed for all of the compounds used, and the effect of organic loading and biological solids (MLVSS) on the rates were studied. The removal rate data for all three compounds were scattered. There are two possible causes for this scatter. First, some of the scatter probably was the result of experimental error. Most of the determinations required sample dilutions before measurement. The precision of some of the

methods used could have also been a factor in the scatter of the data. An even more reasonable answer to the data scatter between batch runs was that a freshly acclimated sludge was used for each run. There was no guarantee that the same genus and species of organisms predominated during each run at a similar loading or that the relative numbers were the same. In fact, any assumptions along this line of thought would be highly suspect. The growth rates among organisms of the same genus vary greatly; therefore, it is doubtful that linear removal rates from two activated sludges would be identical, even under the same experimental conditions.

Finally, it should be noted that the actual magnitudes of the substrate removal rates were very small and the data, although scattered, were always of the same order of magnitude.

Organic loading primarily affected the microbiological properties of the sludge, as other investigators have indicated. Loadings greater than 1.0 lb COD/lb solids·day produced a highly filamentous, poor settling activated sludge. This filamentous sludge was capable of assimilating glucose and phenol at significantly greater rates per unit biological solids than the flocculated, non-filamentous sludge found at low loadings. The unit rate of removal of aniline seemed to be unaffected by the type of sludge.

Since filamentous organisms are known to flourish in industrial wastewaters with high carbohydrate contents, the higher glucose removal rates observed for the filaments might have been expected. No data on phenol removal by filaments have been reported.

The effect of biological solids on substrate removal rates was as expected. Increased biological solids concentration (MLVSS) resulted in increased linear removal rates. Thus, solids (MLVSS) concentration was a good parameter for estimating viable bacteria in the activated sludge system. A given increase in biological solids produced a greater increase in linear removal rate for the filamentous sludges than for the flocculent sludges. The results of the loading and solids studies suggest that filamentous-type sludges can be expected to give higher removal rates for glucose and phenol. If it were possible to provide an economical solids-liquid separation for this type of sludge, it might be desirable to use filamentous organisms to treat wastes high in carbohydrates and phenol.

Two additional substrates, glycine and sodium acetate, were combined with the three compounds previously used in order to observe the substrate removal kinetics in a five substrate system. Batch tests using sludge acclimated to these five substrates indicated that all five were removed linearly. The linear removal rates for glucose, phenol, and aniline were unaffected by the additional two compounds. If one type of organism was using all of the compounds, it would seem likely, if not probable, that some sequential substrate removal would be observed. The simultaneous removal of five compounds by an acclimated activated sludge further substantiates the hypothesis that each compound is utilized by a different type of organism. Another possibility is that a single organism could have a separate permease system for each of the substrates. These permease systems would also enable an organism to utilize all of the simple compounds simultaneously. An identification of all of the organisms present

in the activated sludge system, which is virtually impossible, would be necessary before the correct alternative could be chosen. The most important conclusion which can be drawn from these data is that the removal of simple organic compounds by a sludge acclimated to these compounds is linear and silumtaneous.

A primary objective of this investigation was the comparison of the COD removal from a unit with mixed substrates to the COD removal computed by a summation of the COD removals measured for each of the individual compounds forming the mixture. This sum was computed for each of the sampling times used in the batch studies. The results indicated that COD of the mixed unit could be closely approximated by this method, provided that the non-biodegradable, carryover COD from the mixed unit was accounted for. Comparisons were made for all of the mixed units used in the batch runs. Total organic carbon was also used as an overall parameter of substrate concentration. Predicted and measured TOC for the mixed units agreed very closely.

Several of the COD removal curves for the mixed units closely approximated first order kinetics. However, as was pointed out in a previous mathematical study (40), when three substrates are used, the overall removal is so greatly influenced by the removal of each compound that it is difficult to approximate higher order kinetics by the summation of linear removals of each compound. Only one run was made using five substrates; and although it closely approximated first order COD removal, it would be presumptuous to make any predictions from that data.

The predicted and measured COD and TOC data show that the removal of a mixture of simple organics is actually a linear removal of each of the compounds in the mixture. It was also demonstrated that the removal of the mixture as measured by an overall organic parameter could follow first order kinetics. These findings give a valid explanation for the occurrence of the many types of removal kinetics which are observed for complex wastes. The shape of the removal curve for a complex waste would be dependent upon the removal rates and concentrations of the simple compounds forming the complex waste. The kinetic models which have been used merely represent the fitting of a curve to a discontinuous function. While a given model may fit many complex wastes, it cannot possibly fit them all. Any kinetic model which uses an overall parameter such as COD or BOD can only be expected to represent a few specialized cases.

The concept investigated here would apply to all complex wastes treated by biological means. It has a sound theoretical basis in that linear removal of simple compounds by microorganisms is an established fact, both by pure and heterogeneous bacterial cultures. This theory points out the need for laboratory and pilot plant tests before design of a biological system to treat a specific waste. Since most wastewaters are composed of a vast number of simple compounds which are virtually impossible to measure, this hypothesis could rarely be applied directly. However, it shows the need for considering each wastewater as a separate entity, and cautions against using any kinetic model without prior laboratory tests to determine the validity of the model for the waste under investigation.

The results of this investigation indicate that removal of simple organic compounds by activated sludge is linear to low substrate concentrations. This finding is in accordance with the Michaelis theory of enzyme-substrate interaction which predicts zero-order substrate removal when all of the enzyme sites are saturated. Since it is now known that substrates are transported into microbial cells via permease enzyme systems, these results agree with theoretical considerations.

Table 6-1 gives a comparison of data obtained in this investigation and Michaelis Constants reported by other authors. In this study and several others listed in the table, zero-order substrate removals continued to substrate concentrations below the level of accuracy of the analytical techniques used, thus preventing computation of the actual Michaelis Constants. However, in these cases it was possible to determine the concentration at which linear removal of the simple organics ceased. The Michaelis Constants for these substrates are reported in Table 6-1 as being less than these cutoff values. This procedure at least gives a general order of magnitude for the Michaelis Constants of these compounds.

The data from this investigation compare favorably with that reported by other investigators. Several values of $K_m$ reported in the table seem considerably higher than the majority. Some of these high values were obtained in anaerobic digesters and since anaerobic bacteria are known to have generally lower growth rates than aerobic bacteria, these lower removal rates can be readily explained. The other high $K_m$ values were obtained using $BOD_5$ as a parameter of substrate concentration, which is probably impractical for use in this type of investigation. The low $K_m$ values

Table 6-1

| Substrate | $K_m$[1] (mg/l) | Culture Conditions | Investigator |
|---|---|---|---|
| Glucose | 4 | Aerobic, Pure Culture | Monod (32) |
| Glucose | 0.2 | Activated Sludge | Wuhrmann (44) |
| Glucose | <4 | " " | Wuhrmann (43) |
| Glucose | 133[2] | " " | Tench and Morton (39) |
| Glucose | <13 | " " | This Study |
| Acetate | <20 | " " | Downing (11) |
| Acetate | 259[2] | " " | Tench and Morton (39) |
| Acetate | 154 | Methane Fermentation | Lawrence and McCarty (27) |
| Acetate | <10 | Activated Sludge | This Study |
| Glycine | <8 | " " | Carlson and Polkowski (5) |
| Glycine | <16 | " " | This Study |
| Phenol | <1 | " " | This Study |
| Aniline | <10 | " " | This Study |
| Propionate | 40 | Methane Fermentation | Lawrence and McCarty (27) |
| Butyrate | 7 | " " | " " (27) |
| Aspartic Acid | <4 | Activated Sludge | Wuhrmann (43) |
| Phthalic Acid | 31[2] | " " | Wilson (42) |

[1] $K_m$ values which are listed as being less than the concentrations given in this table indicate that not enough data were available for actual computation of $K_m$. The concentration presented is the point at which zero-order removal ceased. The actual $K_m$ values would be somewhat lower than these concentrations.

[2] as $BOD_5$

reported in this study and most of the other investigations indicate that substrate removal by activated sludge will normally be linear for the substrates and substrate concentrations found in wastewaters.

Temperature and pH were kept constant throughout this investigation. Sodium bicarbonate was added for pH control when it was necessary.

# Chapter 7

## CONCLUSIONS

(1) The simple organic compounds glucose, phenol, and aniline were all removed linearly (zero order) by an activated sludge acclimated to each of the compounds. Variation of the initial concentration of each simple compound had no effect on the removal rate of the compound by a given sludge.

(2) The removal rates of the compounds in a unit containing a mixture of the three were the same as the rates found when each compound was tested alone, using the same sludge for both tests. This simultaneous and non-preferential removal of the three compounds indicates that a different type of organism was probably responsible for the removal of each simple substrate. Additional investigations using two more linearly removed organics, glycine and acetate, showed no effect on the removal rates of the original three components.

(3) The predominant type of microorganism present in the continuous activated sludge unit was determined by the organic loading level on the unit. The filamentous sludge which was found at high loadings was capable of assimilating glucose and phenol at significantly greater rates per unit biological solids than the flocculent sludges found at low and intermediate organic loadings. Aniline removal rates seemed to be unaffected by sludge type.

(4) The linear removal rate for each compound increased with increasing biological solids concentration (MLVSS). When glucose or phenol substrates were used, the net increase in removal rate for a given solids

increase was greater for the filamentous sludge than for the flocculent sludge. Insufficient data was available to establish this relationship definitely for aniline, but it appeared that this effect was not as significant for aniline as for the other two substrates.

(5) Overall organic removal, as measured by COD or TOC, from a mixture of three or five linearly-removed simple compounds by an activated sludge acclimated to these compounds is actually a summation of the removals of each of the simple compounds. The removal of these mixtures can approximate first order kinetics. This observation offers an explanation for the many types of removal kinetics observed during the biological treatment of complex wastes.

(6) High initial phenol concentrations in a batch system can inhibit the removal of other simple organics in a complex waste. The removal of the compounds remained linear, but proceeded at a lower rate than when the inhibition was absent. This observation appears to be an inhibition of the organisms which utilize the other organics in the mixture. Phenol removal is also inhibited by high initial concentrations. This inhibition of the phenol-utilizing bacteria is distinguished by a short lag phase followed by linear removal of the phenol. The phenol assimilation capacity of the system is unaffected by this lag.

# APPENDIX A

# APPENDIX A-1

## Batch Runs 7/22/66

| Continuous Unit Data: | | Influent mg/l | Effluent mg/l | |
|---|---|---|---|---|
| Averages | | | | |
| | COD | 610 | 63 | MLSS = 2600 |
| | Glucose | 200 | 10 | MLVSS = 2150 |
| | Phenol | 100 | 3 | $L_f$ = 1.2 |
| | Butanal | 100 | 1 | |

Batch Data (mg/l unless otherwise indicated)

| Unit No. | Avg. MLVSS | Time (hr) | Glucose | Phenol | Butanal | COD |
|---|---|---|---|---|---|---|
| A | | 0 | | 88 | | 255 |
| | | 0.5 | | 72 | | 220 |
| | | 1 | | 60 | | 153 |
| | | 2 | | 34 | | 122 |
| | 1990 | 3 | | 4 | | 48 |
| | | 4 | | 5 | | 61 |
| | | 5 | | 3 | | 30 |
| | | 6 | | 5 | | 35 |
| | | 7 | | 4 | | 74 |
| B | | 0 | | | 100 | 116 |
| | | 0.5 | Oxidized and | | - | 57 |
| | | 1 | stripped from | | - | 97 |
| | | 2 | solution by | | - | 78 |
| | | 3 | aeration | | - | 32 |
| | | 4 | | | - | 44 |
| | | 5 | | | - | 40 |
| | | 6 | | | - | 50 |
| | | 7 | | | - | 50 |
| C | | 0 | 200 | | | 218 |
| | | 0.5 | 100 | | | 156 |
| | | 1 | 36 | | | 60 |
| | | 2 | 14 | | | 46 |
| | 1980 | 3 | 15 | | | 46 |
| | | 4 | 12 | | | 42 |
| | | 5 | 9 | | | 40 |
| | | 6 | 11 | | | 40 |
| | | 7 | 11 | | | 40 |

APPENDIX A-1 (Contd.)

Batch Runs 7/22/66

Batch Data (Contd.)

| Unit No. | Avg. MLVSS | Time (hr) | Glucose | Phenol | Butanal | COD |
|---|---|---|---|---|---|---|
| D | | 0 | 195 | 96 | 100 | 472 |
| | | 0.5 | 100 | 88 | - | 386 |
| | | 1 | 10 | - | - | 201 |
| | | 2 | 8 | 46 | - | 158 |
| | 1900 | 3 | 10 | 10 | - | 82 |
| | | 4 | 8 | 5 | - | 46 |
| | | 5 | 9 | 4 | - | 53 |
| | | 6 | 10 | 7 | - | 46 |
| | | 7 | 10 | 5 | - | 64 |

BATCH DATA 7-22-66

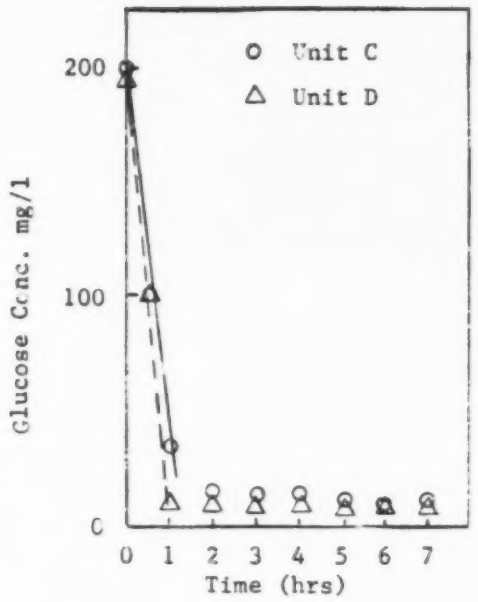


Figure A-1(a)

Glucose Removal

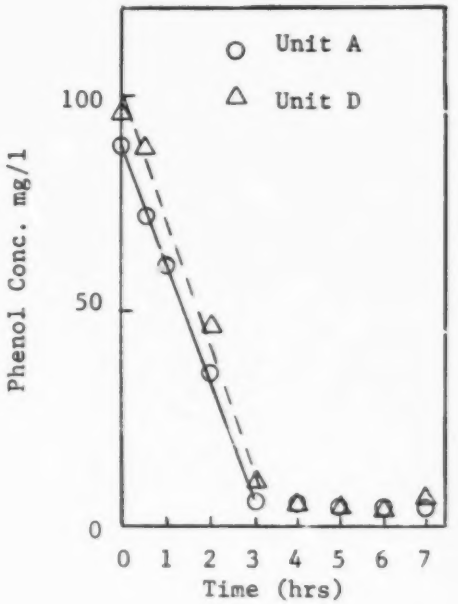


Figure A-1(b)

Phenol Removal

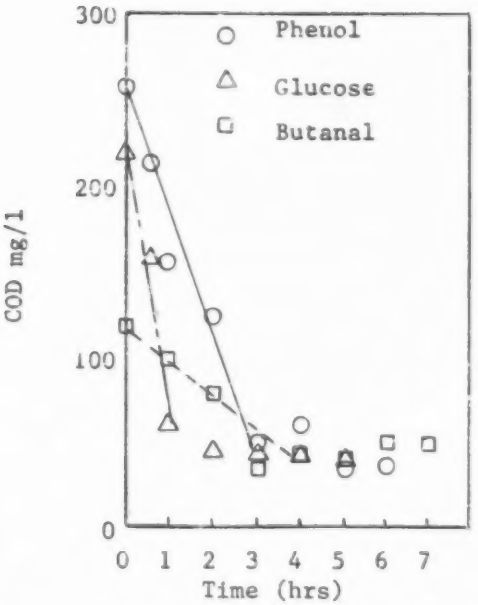


Figure A-1 (c)

COD Removal - Single Substrates

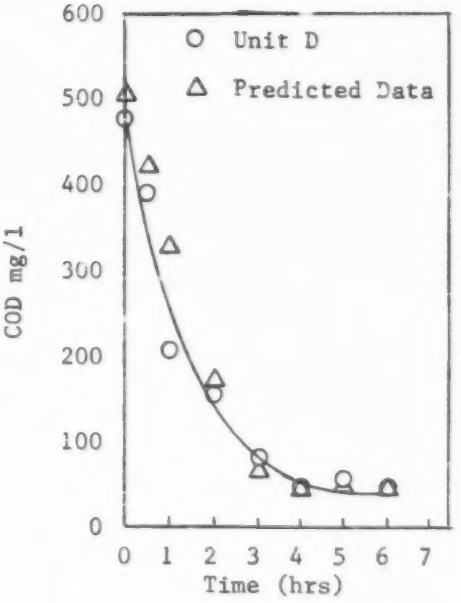


Figure A-1 (d)

COD Removal - Mixed Unit

# APPENDIX A-2

## Batch Runs 9/16/66

**Continuous Unit Data:**

| Averages | Influent mg/l | Effluent mg/l | |
|---|---|---|---|
| COD | 316 | 22 | MLSS = 3230 |
| Glucose | 100 | 4 | MLVSS = 2620 |
| Phenol | 50 | <1 | $L_f$ = 0.3 |
| Aniline | 50 | <1 | |

**Batch Data** (mg/l unless otherwise indicated)

| Unit No. | Avg. MLVSS | Time (hr) | Glucose | Phenol | Aniline | COD |
|---|---|---|---|---|---|---|
| A | | 0 | 73 | | | 162 |
| | | 0.5 | 26 | | | 51 |
| | 2580 | 1.0 | 8 | | | 36 |
| | | 1.5 | 5 | | | 38 |
| | | 2 | 3 | | | (90) |
| | | 3 | 3 | | | 36 |
| B | | 0 | | 45 | | 132 |
| | | 0.5 | | 42 | | 128 |
| | 2540 | 1.0 | | 39 | | 137 |
| | | 1.5 | | 36 | | 108 |
| | | 2 | | 33 | | 93 |
| | | 3 | | 23 | | 76 |
| C | | 0 | | | 36 | 145 |
| | | 0.5 | | | 25 | 101 |
| | 2540 | 1.0 | | | 15 | 65 |
| | | 1.5 | | | 7 | 43 |
| | | 2 | | | 1 | 40 |
| | | 3 | | | <1 | 46 |
| D | | 0 | 89 | 46 | 39 | 338 |
| | | 0.5 | 45 | 44 | 30 | 252 |
| | 2460 | 1.0 | 6 | 44 | 21 | 162 |
| | | 1.5 | - | - | 5 | 129 |
| | | 2 | 4 | 38 | - | 124 |
| | | 3 | 3 | 27 | - | 80 |

BATCH DATA 9-16-66

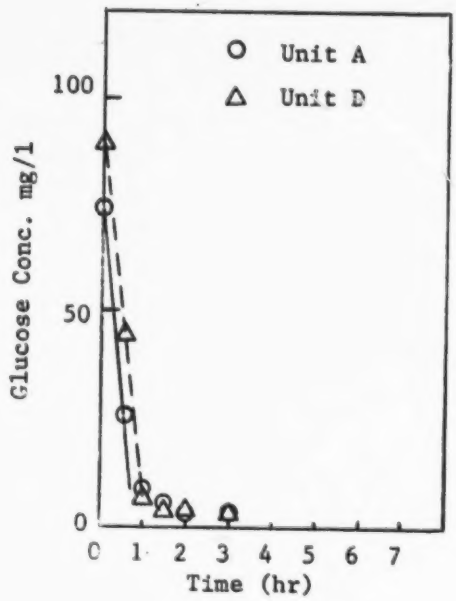


Figure A-2 (a)

Glucose Removal

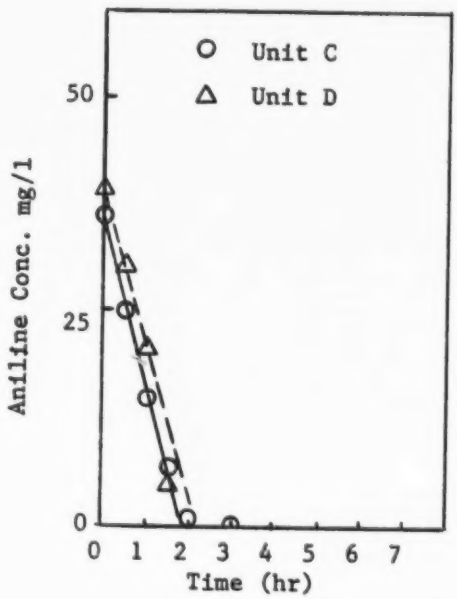


Figure A-2(b)

Aniline Removal

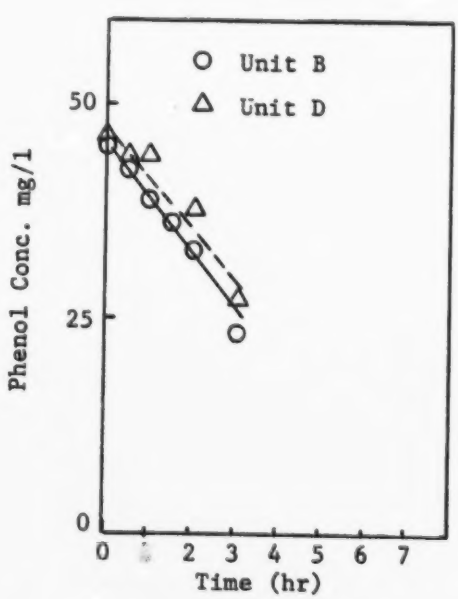


Figure A-2(c)

Phenol Removal

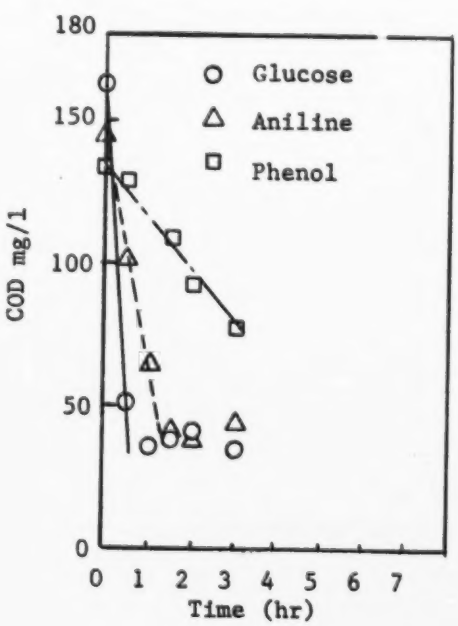


Figure A-2(d)

COD Removal - Single Substrates

BATCH DATA 9-16-66

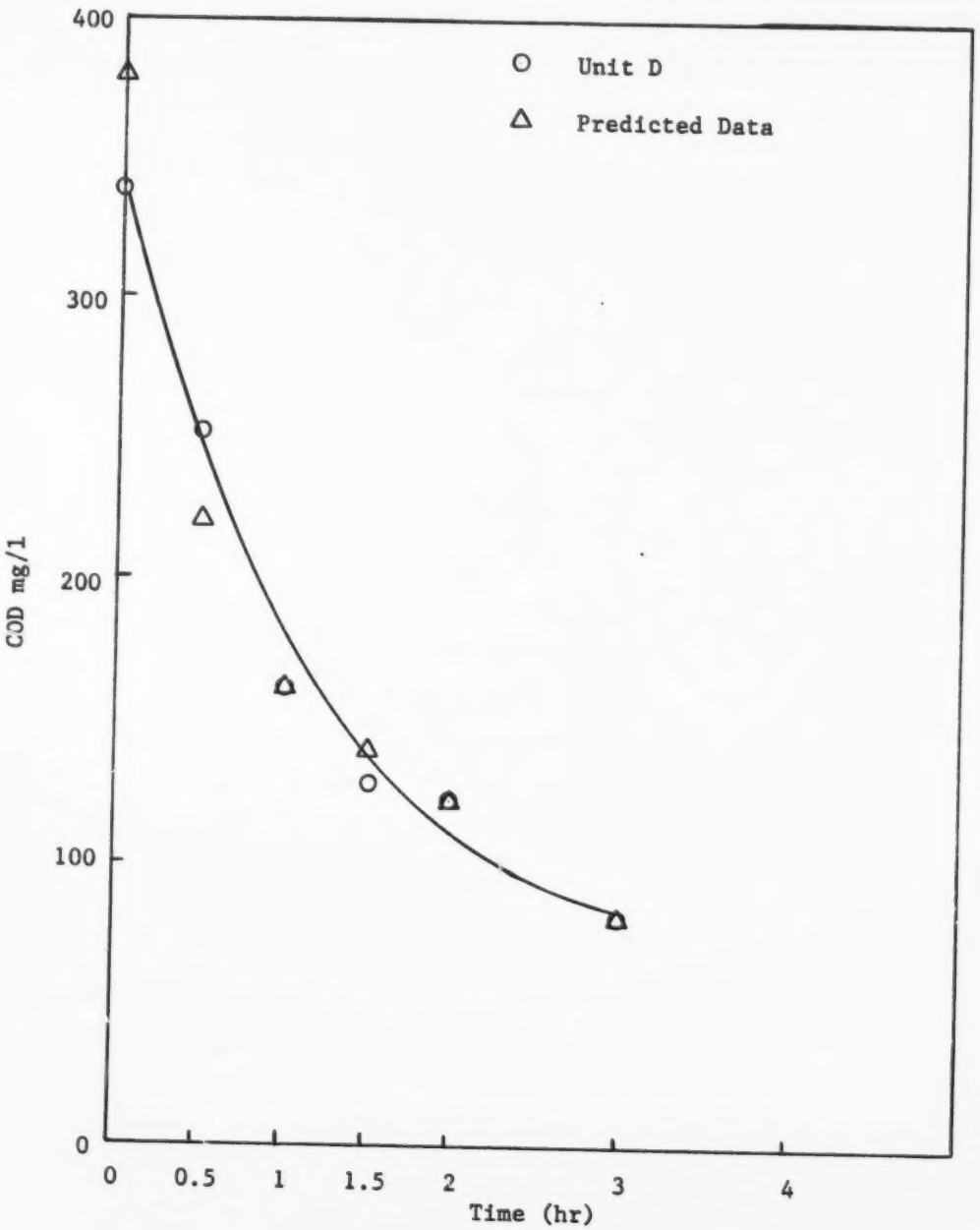


Figure A-2 (e)

COD Removal - Mixed
Batch Unit

# APPENDIX A-3

## Batch Runs 10/20/66

**Continuous Unit Data:**

| Averages | Influent mg/l | Effluent mg/l | |
|---|---|---|---|
| COD | 980 | 30 | MLSS = 3160 |
| Glucose | 300 | 15 | MLVSS = 2930 |
| Phenol | 150 | <1 | $L_f$ = 1.1 |
| Aniline | 150 | <1 | |

**Batch Data** (in mg/l unless otherwise indicated)

| Unit No. | Avg. MLVSS | Time (hr) | Glucose | Phenol | Aniline | COD |
|---|---|---|---|---|---|---|
| A | | 0 | | 140 | | 354 |
| | | 1 | | 82 | | 231 |
| | 2990 | 2 | | 4 | | 53 |
| | | 3 | | <1 | | 11 |
| | | 4 | | <1 | | 11 |
| | | 5 | | <1 | | 50 |
| B | | 0 | | | 156 | 398 |
| | | 1 | | | 122 | 367 |
| | 2960 | 2 | | | 121 | 345 |
| | | 3 | | | 90 | 295 |
| | | 4 | | | 66 | 301 |
| | | 5 | | | 60 | 227 |
| C | | 0 | 240 | | | 268 |
| | | 1 | 34 | | | 62 |
| | 2820 | 2 | 14 | | | 57 |
| | | 3 | 15 | | | 60 |
| | | 4 | 17 | | | 20 |
| | | 5 | 15 | | | 55 |
| D | | 0 | 280 | 147 | 168 | 916 |
| | | 1 | 28 | 102 | 148 | 564 |
| | 2870 | 2 | 32 | 64 | 112 | 418 |
| | | 3 | 27 | 26 | 80 | 288 |
| | | 4 | 20 | 5 | 42 | 266 |
| | | 5 | 18 | <1 | 18 | 123 |

APPENDIX A-3 (Contd.)

Batch Runs 10/20/66

Batch Data (Contd.)

| Unit No. | Avg. MLVSS | Time (hr) | Glucose | Phenol | Aniline | COD |
|---|---|---|---|---|---|---|
| E | | 0 | | 88 | | 264 |
| | | 1 | | <1 | | 94 |
| | 2950 | 2 | | <1 | | 27 |
| | | 3 | | <1 | | 42 |
| | | 4 | | <1 | | 38 |
| | | 5 | | <1 | | 36 |
| F | | 0 | | | 72 | 211 |
| | | 1 | | | 48 | 163 |
| | 2990 | 2 | | | 25 | 110 |
| | | 3 | | | 7 | 88 |
| | | 4 | | | 4 | 84 |
| | | 5 | | | 5 | 75 |
| G | | 0 | 120 | | | 142 |
| | | 1 | 48 | | | 75 |
| | 2920 | 2 | 18 | | | 39 |
| | | 3 | 14 | | | 41 |
| | | 4 | 17 | | | 30 |
| | | 5 | 17 | | | 43 |

BATCH DATA 10-22-66

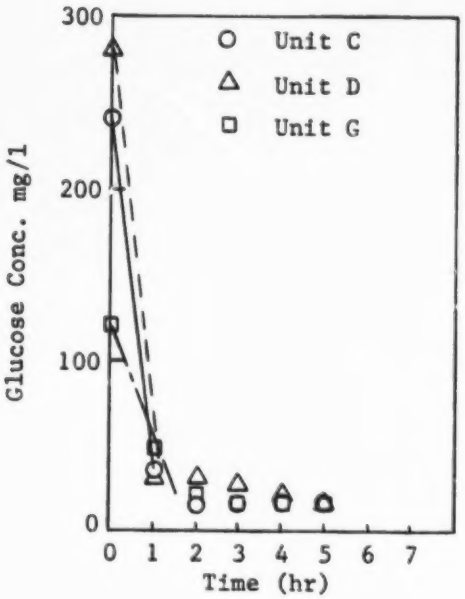


Figure A-3(a)

Glucose Removal

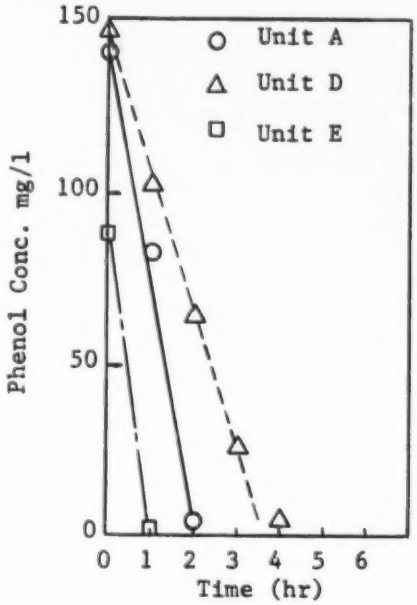


Figure A-3(b)

Phenol Removal

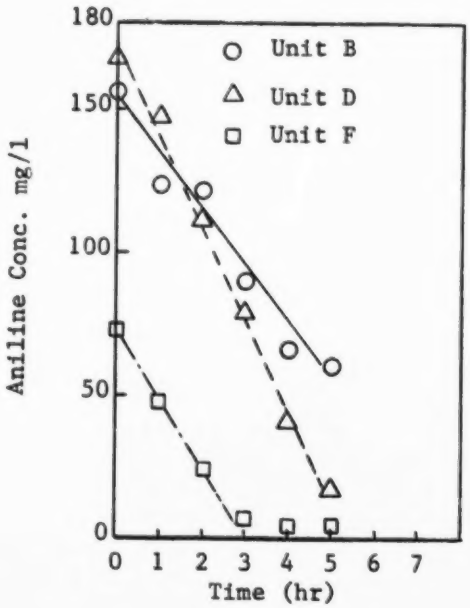


Figure A-3(c)

Aniline Removal

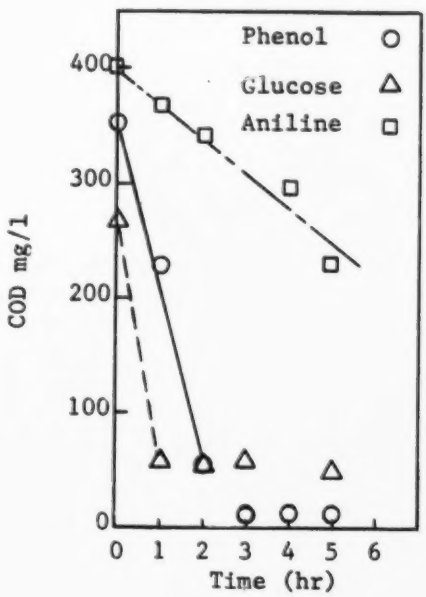


Figure A-3 (d)

COD Removal - Single Substrates
Units A, B, C

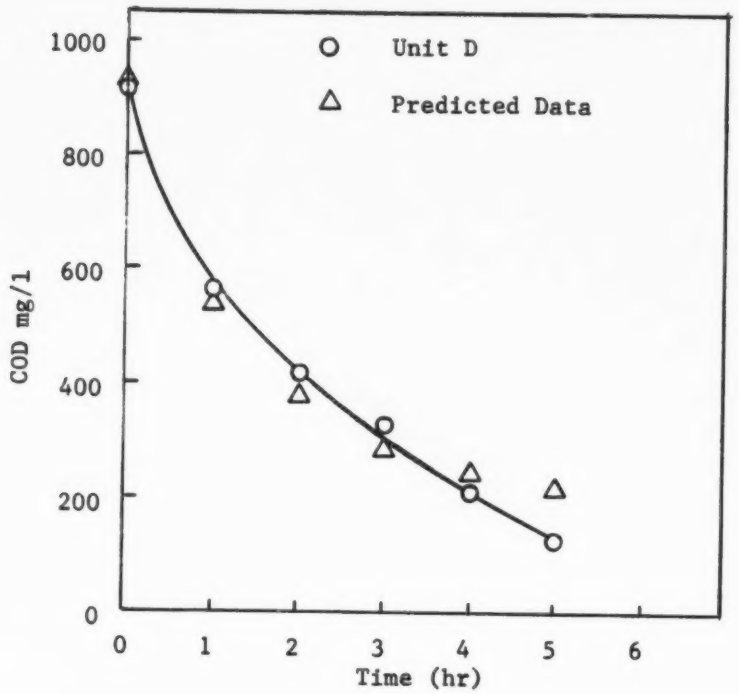


Figure A-3(e) COD Removal - Mixed Unit

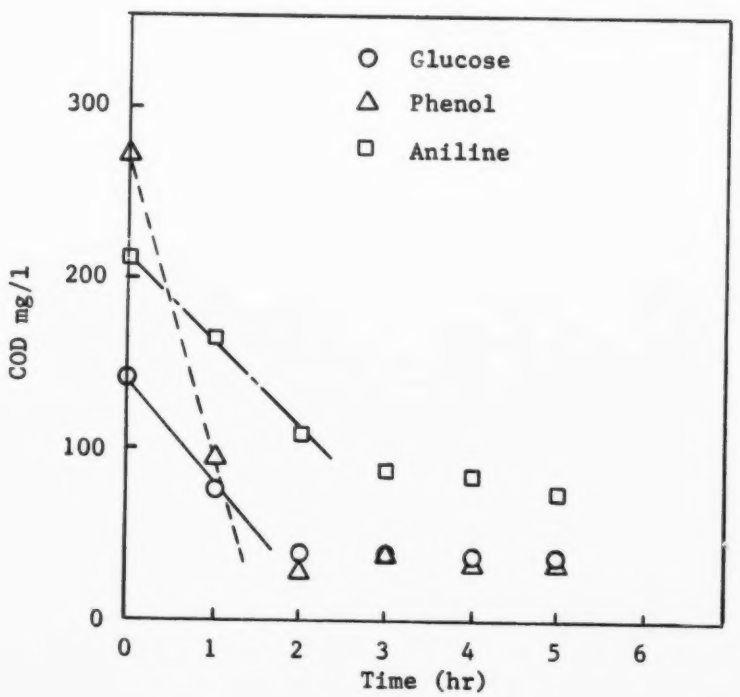


Figure A-3(f)

COD Removal - Single Substrates
Units E, F, G

# APPENDIX A-4

## Batch Runs 2/9/67

| Continuous Unit Data: | | Influent mg/l | Effluent mg/l | |
|---|---|---|---|---|
| Averages | | | | |
| | COD | 330 | 30 | MLSS = 2520 |
| | Glucose | 100 | 4 | MLVSS = 1990 |
| | Phenol | 50 | <1 | $L_f$ = 0.25 |
| | Aniline | 50 | <1 | |

**Batch Data** (in mg/l unless otherwise indicated)

| Unit No. | Avg. MLVSS | Time (hr) | Glucose | Phenol | Aniline | COD |
|---|---|---|---|---|---|---|
| A | | 0 | 97 | | | 135 |
| | | 1 | 55 | | | 55 |
| | | 2 | 8 | | | 30 |
| | | 3 | 9 | | | 30 |
| | 1610 | 4 | 5 | | | 27 |
| | | 5 | 8 | | | 27 |
| | | 6 | 6 | | | 23 |
| | | 7 | 7 | | | 27 |
| | | 24 | 5 | | | 23 |
| B | | 0 | | 26 | | 130 |
| | | 1 | | 22 | | 115 |
| | | 2 | | 21 | | 105 |
| | | 3 | | 14 | | 75 |
| | 1560 | 4 | | 11 | | 65 |
| | | 5 | | 3 | | 30 |
| | | 6 | | <1 | | 25 |
| | | 7 | | <1 | | 25 |
| | | 24 | | <1 | | 20 |
| C | | 0 | | | 51 | 165 |
| | | 1 | | | 42 | 145 |
| | | 2 | | | 36 | 105 |
| | | 3 | | | 24 | 90 |
| | 1380 | 4 | | | 12 | 60 |
| | | 5 | | | 9 | 40 |
| | | 6 | | | 8 | 40 |
| | | 7 | | | 8 | 35 |
| | | 24 | | | 7 | 35 |

APPENDIX A-4 (Contd.)

Batch Runs 2/9/67

Batch Data (Contd.)

| Unit No. | Avg. MLVSS | Time (hr) | Glucose | Phenol | Aniline | COD |
|---|---|---|---|---|---|---|
| D | | 0 | 104 | 62 | 29 | 370 |
| | | 1 | 68 | 61 | 21 | 330 |
| | | 2 | 28 | 52 | 14 | 225 |
| | | 3 | - | 44 | 5 | 185 |
| | 1490 | 4 | 25 | 34 | 4 | 160 |
| | | 5 | 18 | 27 | 6 | 125 |
| | | 6 | 24 | 21 | 3 | 120 |
| | | 7 | 32 | 18 | 3 | 90 |
| | | 24 | 8 | 1 | 0 | 65 |
| E | | 0 | 352 | | | 315 |
| | | 1 | 284 | | | 254 |
| | | 2 | 260 | | | 225 |
| | | 3 | 156 | | | 185 |
| | 1650 | 4 | 98 | | | 135 |
| | | 5 | 51 | | | 80 |
| | | 6 | 6 | | | 30 |
| | | 7 | 7 | | | 30 |
| | | 24 | 5 | | | 50 |
| F | 1390 | 0 | | 102 | | 385 |
| | - Unit | 1 | | 100 | | 350 |
| | shocked | 2 | | 102 | | 340 |
| | solids | 3 | | 104 | | 330 |
| | dropping | 4 | | 104 | | 355 |
| | | 5 | | 104 | | 325 |
| | | 6 | | 107 | | 310 |
| | | 7 | | 100 | | 318 |
| | | 24 | | <1 | | 97 |
| G | | 0 | | | 66 | 445 |
| | | 1 | | | 50 | 363 |
| | | 2 | | | 50 | 370 |
| | | 3 | | | 43 | 350 |
| | 1530 | 4 | | | 43 | 305 |
| | | 5 | | | 35 | 295 |
| | | 6 | | | 31 | 275 |
| | | 7 | | | 31 | 235 |
| | | 24 | | | 27 | 110 |

BATCH DATA 2-9-67

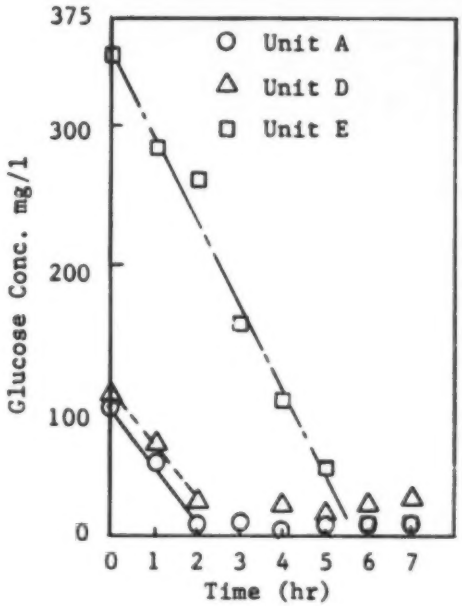


Figure A-4(a)

Glucose Removal

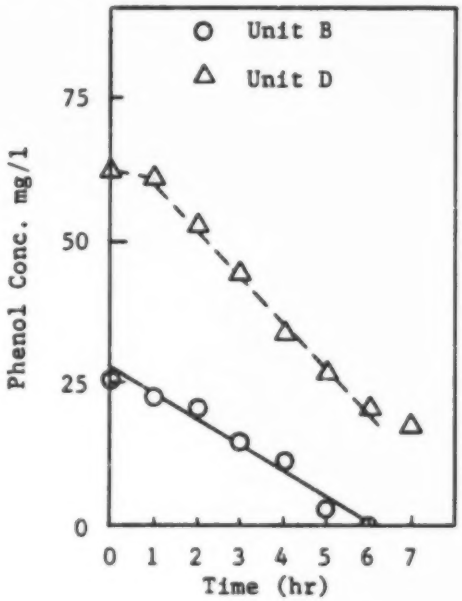


Figure A-4(b)

Phenol Removal

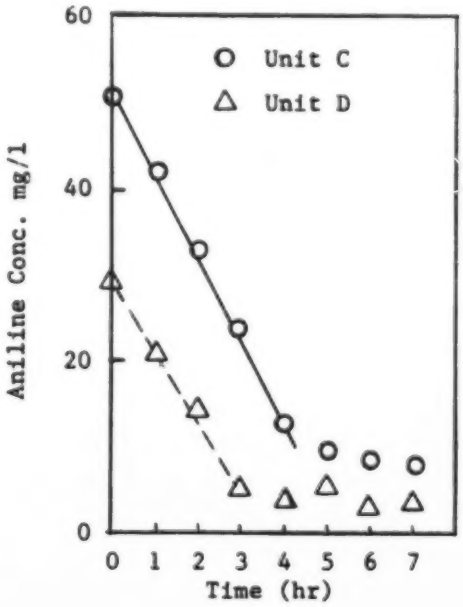


Figure A-4(c)
Aniline Removal

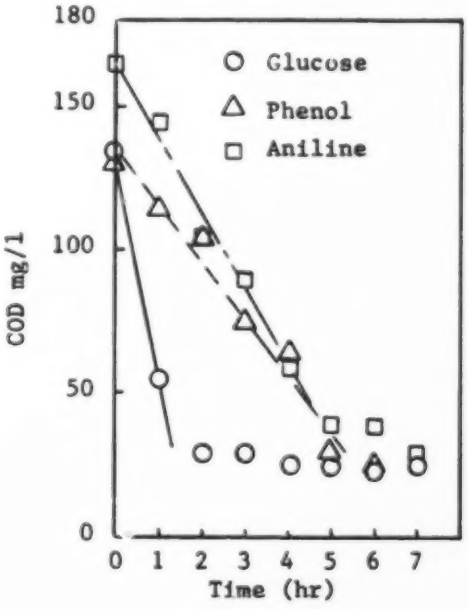


Figure A-4(d)

COD Removal - Single
Substrates - Units A, B, C

# APPENDIX A-5

## Batch Runs 3/9/67

**Continuous Unit Data:**

| Averages | Influent mg/l | Effluent mg/l | |
|---|---|---|---|
| COD | 330 | 48 | MLSS = 3470 |
| Glucose | 100 | 5 | MLVSS = 2800 |
| Phenol | 50 | 0.3 | $L_f$ = 0.2 |
| Aniline | 50 | <1 | |

**Batch Data** (in mg/l unless otherwise indicated)

| Unit No. | Avg. MLVSS | Time (hr) | Glucose | Phenol | Aniline | COD | $BOD_5$ |
|---|---|---|---|---|---|---|---|
| A | | 0 | 100 | | | 110 | |
| | | 1 | 15 | | | 30 | |
| | | 2 | 3 | | | 30 | |
| | | 3 | 3 | | | 30 | |
| | 2660 | 4 | 3 | | | 30 | |
| | | 5 | 3 | | | 30 | |
| | | 6 | 3 | | | 30 | |
| | | 7 | 3 | | | 27 | |
| | | 24 | 7 | | | 23 | 8.7 |
| B | | 0 | | 46 | 53 | 235 | |
| | | 1 | | 39 | 46 | 188 | |
| | | 2 | | 29 | 35 | 136 | |
| | | 3 | | 15 | 15 | 65 | |
| | 2600 | 4 | | <1 | 2 | 25 | |
| | | 5 | | <1 | 1 | 25 | |
| | | 6 | | <1 | 2 | 25 | |
| | | 7 | | <1 | <1 | 30 | |
| | | 24 | | <1 | <1 | 25 | 2.1 |
| C | | 0 | | | 50 | 135 | |
| | | 1 | | | 39 | 70 | |
| | | 2 | | | 17 | 60 | |
| | | 3 | | | 3 | 30 | |
| | 2700 | 4 | | | <1 | 30 | |
| | | 5 | | | <1 | 30 | |
| | | 6 | | | <1 | 10 | |
| | | 7 | | | <1 | 10 | |
| | | 24 | | | <1 | 10 | 1.8 |

APPENDIX A-5 (Contd.)

Batch Runs 3/9/67

Batch Data (Contd.)

| Unit No. | Avg. MLVSS | Time (hr) | Glucose | Phenol | Aniline | COD | $BOD_5$ |
|---|---|---|---|---|---|---|---|
| D | | 0 | 102 | 52 | 51 | 300 | |
| | | 1 | 35 | 41 | 45 | 220 | |
| | | 2 | 7 | 35 | 36 | 155 | |
| | | 3 | 9 | 20 | 21 | 98 | |
| | 2470 | 4 | 5 | 1 | 7 | 30 | |
| | | 5 | 5 | <1 | 1 | 30 | |
| | | 6 | 5 | <1 | <1 | 30 | |
| | | 7 | 5 | <1 | <1 | 25 | |
| | | 24 | 5 | <1 | <1 | 30 | |
| E | | 0 | 50 | 23 | 53 | 212 | |
| | | 1 | 6 | 14 | 43 | 115 | |
| | | 2 | 3 | <1 | 19 | 50 | |
| | | 3 | 5 | <1 | 6 | 30 | |
| | 2750 | 4 | 5 | <1 | 2 | 30 | |
| | | 5 | 5 | <1 | 1 | 30 | |
| | | 6 | 5 | <1 | 1 | 30 | |
| | | 7 | 5 | <1 | 1 | 30 | |
| | | 24 | 5 | <1 | 3 | 25 | |

BATCH DATA 3-9-67

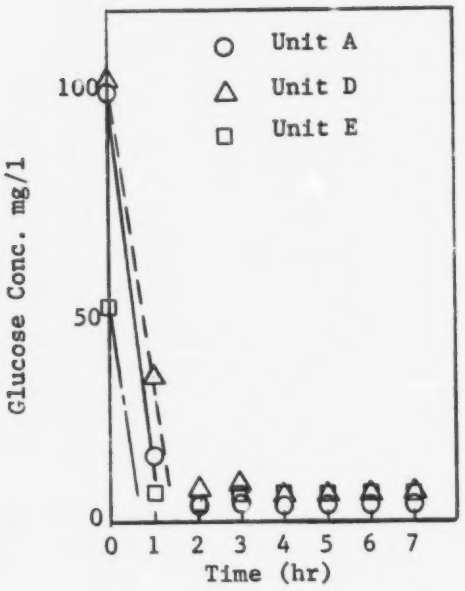


Figure A-5(a)

Glucose Removal

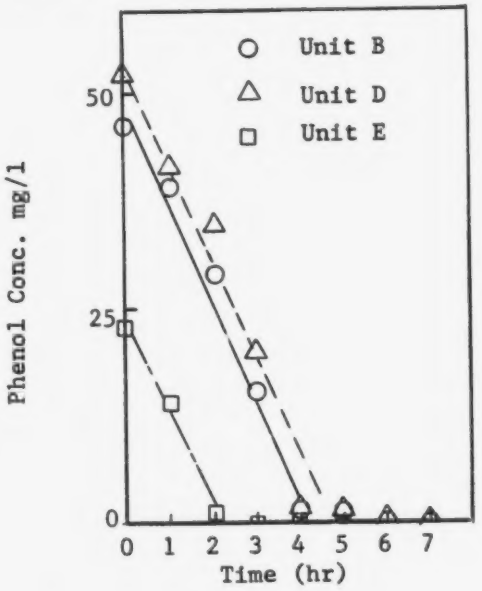


Figure A-5(b)

Phenol Removal

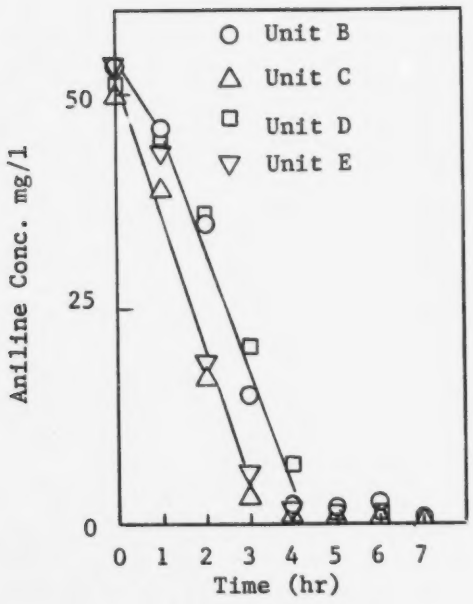


Figure A-5(c)

Aniline Removal

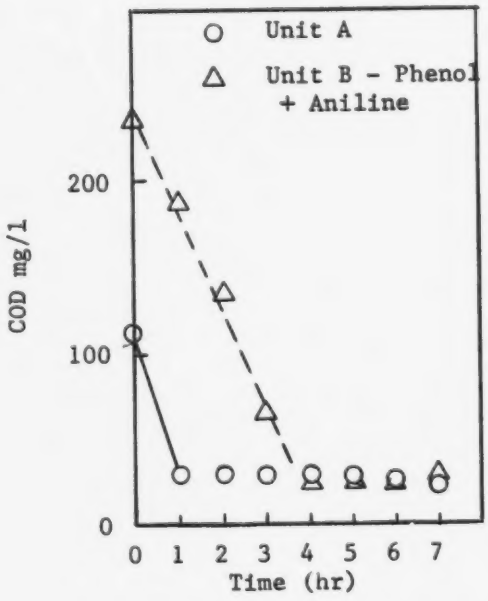


Figure A-5(d)

COD Removal - Single Substrates

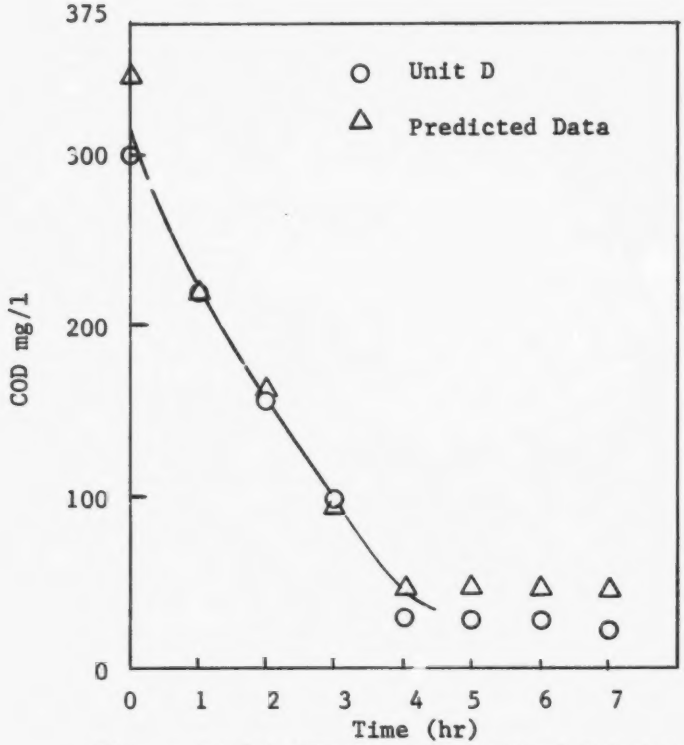


Figure A-5(e) COD Removal - Mixed Substrates

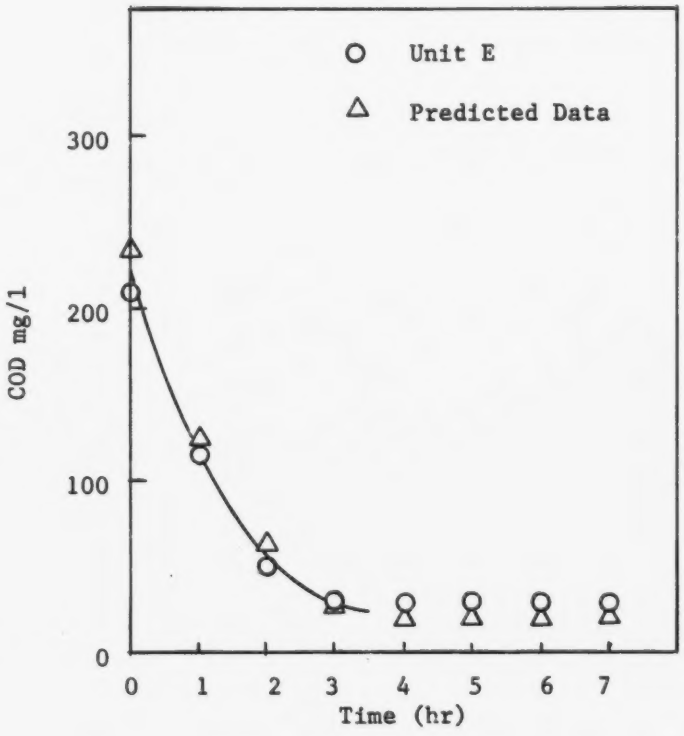


Figure A-5(f) COD Removal - Mixed Substrates

# APPENDIX A-6

## Batch Runs 4/6/67

| Continuous Unit Data: | | Influent mg/l | Effluent mg/l | |
|---|---|---|---|---|
| Averages | | | | |
| | COD | 980 | 80 | MLSS = 5015 |
| | Glucose | 300 | 9 | MLVSS = 4080 |
| | Phenol | 150 | <1 | $L_f$ = 0.42 |
| | Aniline | 150 | <1 | |

Batch Data (in mg/l unless otherwise indicated)

| Unit No. | Avg. MLVSS | Time (hr) | Glucose | Phenol | Aniline | COD | $BOD_5$ |
|---|---|---|---|---|---|---|---|
| A | | 0 | 316 | | | 390 | |
| | | 1 | 172 | | | 210 | |
| | | 2 | 46 | | | 110 | |
| | | 3 | 14 | | | 70 | |
| | 3700 | 4 | 11 | | | 90 | |
| | | 5 | 9 | | | 80 | |
| | | 6 | (23) | | | 80 | |
| | | 7 | 7 | | | 80 | |
| | | 24 | 13 | | | 110 | 41 |
| B | | 0 | | 75 | | 440 | |
| | | 1 | | 57 | | 360 | |
| | | 2 | | 47 | | 350 | |
| | | 3 | | 29 | | 240 | |
| | 3550 | 4 | | 1 | | 110 | |
| | | 5 | | <1 | | 90 | |
| | | 6 | | <1 | | 90 | |
| | | 7 | | <1 | | 90 | |
| | | 24 | | <1 | | 90 | 43 |
| C | | 0 | | | 150 | 360 | |
| | | 1 | | | - | 270 | |
| | | 2 | | | - | 170 | |
| | | 3 | | | - | 90 | |
| | 3600 | 4 | | | - | 95 | |
| | | 5 | | | - | 80 | |
| | | 6 | | | - | 90 | |
| | | 7 | | | - | 90 | |
| | | 24 | | | - | 90 | 43 |

APPENDIX A-6 (Contd.)

Batch Runs 4/6/67

Batch Data (Contd.)

| Unit No. | Avg. MLVSS | Time (hr) | Glucose | Phenol | Aniline | COD | $BOD_5$ |
|---|---|---|---|---|---|---|---|
| D | | 0 | 324 | 150 | 150 | 980 | |
| | | 1 | 212 | 137 | - | 790 | |
| | | 2 | 104 | 107 | - | 610 | |
| | | 3 | 24 | 68 | - | 400 | |
| | 3720 | 4 | 30 | 46 | - | 300 | |
| | | 5 | 18 | 44 | - | 220 | |
| | | 6 | 11 | 1 | - | 100 | |
| | | 7 | 12 | <1 | - | 100 | |
| | | 24 | 14 | <1 | - | 107 | |
| E | | 0 | 100 | 93 | 100 | 600 | |
| | | 1 | 24 | 68 | - | 370 | |
| | | 2 | 22 | 34 | - | 130 | |
| | | 3 | 13 | <1 | - | 80 | |
| | 3600 | 4 | 14 | <1 | - | 90 | |
| | | 5 | 14 | <1 | - | 80 | |
| | | 6 | 12 | <1 | - | 90 | |
| | | 7 | 12 | <1 | - | 90 | |
| | | 24 | 18 | <1 | - | 100 | |

BATCH DATA 4-6-67

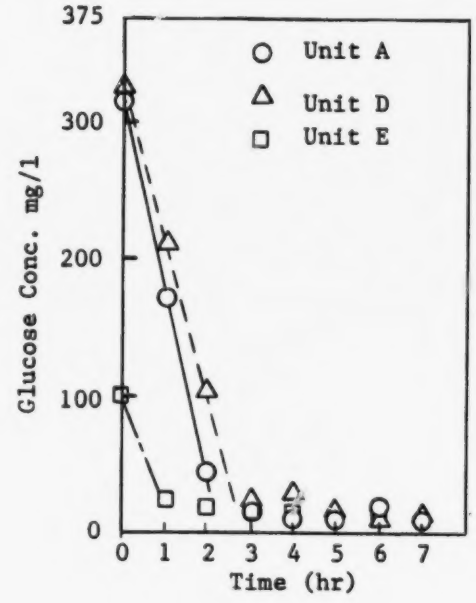


Figure A-6(a)

Glucose Removal

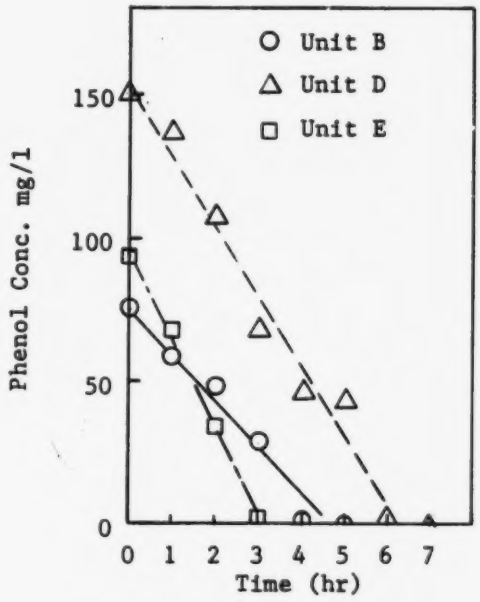


Figure A-6(b)

Phenol Removal

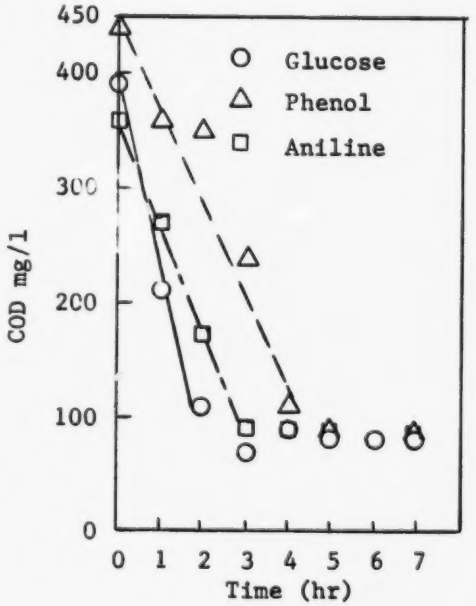


Figure A-6(c)

COD Removal - Single Substrates

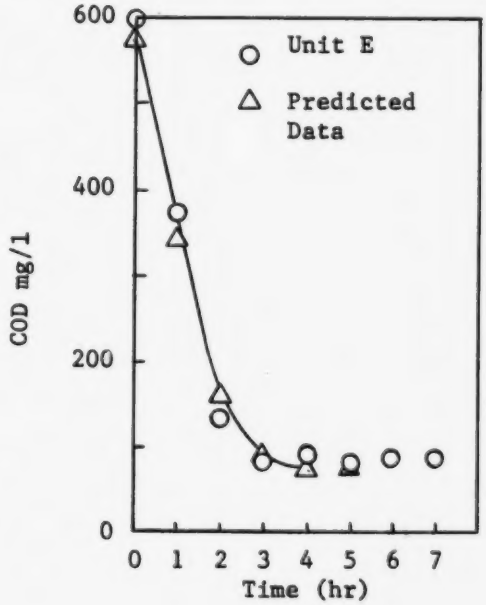


Figure A-6(d)

COD Removal - Mixed Substrates

# APPENDIX A-7

## Batch Runs 4/25/67

**Continuous Unit Data:**

| Averages | Influent mg/l | Effluent mg/l | |
|---|---|---|---|
| COD | 680 | 46 | MLSS = 3020 |
| Glucose | 200 | 7 | MLVSS = 2500 |
| Phenol | 100 | <1 | $L_f$ = 0.52 |
| Aniline | 100 | <1 | |

**Batch Data** (in mg/l unless otherwise indicated)

| Unit No. | Avg. MLVSS | Time (hr) | Glucose | Phenol | Aniline | COD | $BOD_5$ |
|---|---|---|---|---|---|---|---|
| A | | 0 | 174 | | | 235 | |
| | | 1 | 74 | | | 120 | |
| | | 2 | 8 | | | 50 | |
| | | 3 | 7 | | | 50 | |
| | 2330 | 4 | 10 | | | 50 | |
| | | 5 | 10 | | | 60 | |
| | | 6 | 10 | | | 50 | |
| | | 7 | 10 | | | 50 | 5.4 |
| | | 24 | 16 | | | 50 | 3.8 |
| B | | 0 | | 46 | | 280 | |
| | | 1 | | 36 | | 265 | |
| | | 2 | | 24 | | 205 | |
| | | 3 | | <1 | | 65 | |
| | 2370 | 4 | | <1 | | 70 | |
| | | 5 | | <1 | | 60 | |
| | | 6 | | <1 | | 60 | |
| | | 7 | | <1 | | 60 | 6.9 |
| | | 24 | | <1 | | 70 | 6.1 |
| C | | 0 | | | 152 | 240 | |
| | | 1 | | | 100 | 130 | |
| | | 2 | | | 36 | 50 | |
| | | 3 | | | 24 | 50 | |
| | 2300 | 4 | | | 23 | 50 | |
| | | 5 | | | 18 | 40 | |
| | | 6 | | | 16 | 50 | |
| | | 7 | | | 15 | 50 | 8.1 |
| | | 24 | | | 10 | 50 | 5.2 |

APPENDIX A-7 (Contd.)

Batch Runs 4/25/67

Batch Data (Contd.)

| Unit No. | Avg. MLVSS | Time (hr) | Glucose | Phenol | Aniline | COD | $BOD_5$ |
|---|---|---|---|---|---|---|---|
| D | | 0 | 174 | 98 | 100 | 590 | |
| | | 1 | 102 | 72 | - | 425 | |
| | | 2 | 46 | 36 | - | 240 | |
| | | 3 | 16 | 15 | - | 135 | |
| | 2480 | 4 | 11 | <1 | - | 60 | |
| | | 5 | 8 | <1 | - | 40 | |
| | | 6 | 9 | <1 | - | 45 | |
| | | 7 | 9 | <1 | - | 60 | |
| | | 24 | 9 | <1 | - | 60 | |
| E | | 0 | 296 | 74 | 50 | 665 | |
| | | 1 | 174 | 39 | - | 430 | |
| | | 2 | 112 | 19 | - | 285 | |
| | | 3 | 39 | 1 | - | 90 | |
| | | 4 | 11 | <1 | - | 50 | |
| | 2460 | 5 | 10 | <1 | - | 50 | |
| | | 6 | 10 | <1 | - | 50 | |
| | | 7 | 9 | <1 | - | 50 | |
| | | 24 | 9 | <1 | - | 55 | |

BATCH DATA 4-25-67

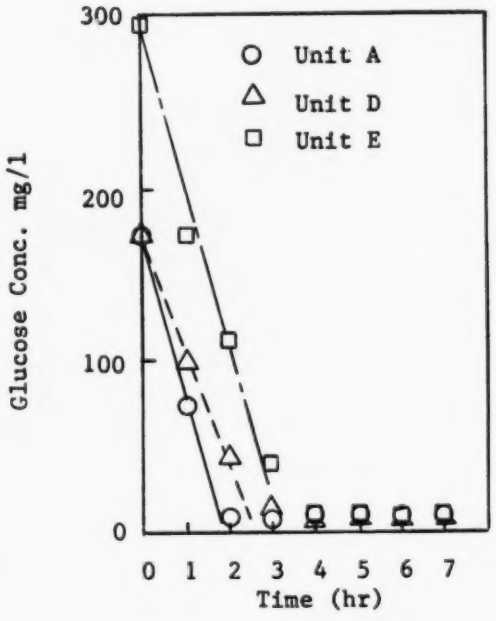


Figure A-7(a)

Glucose Removal

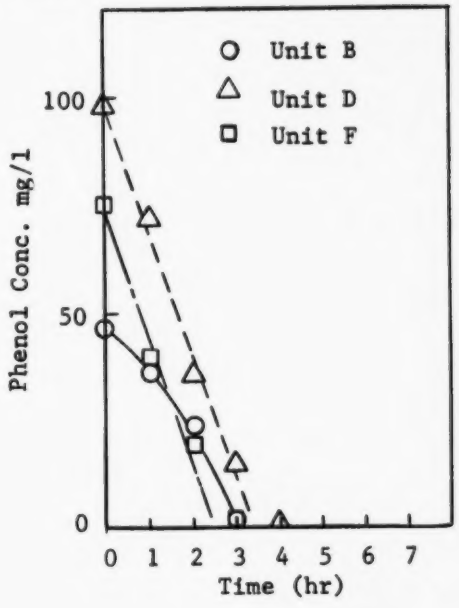


Figure A-7(b)

Phenol Removal

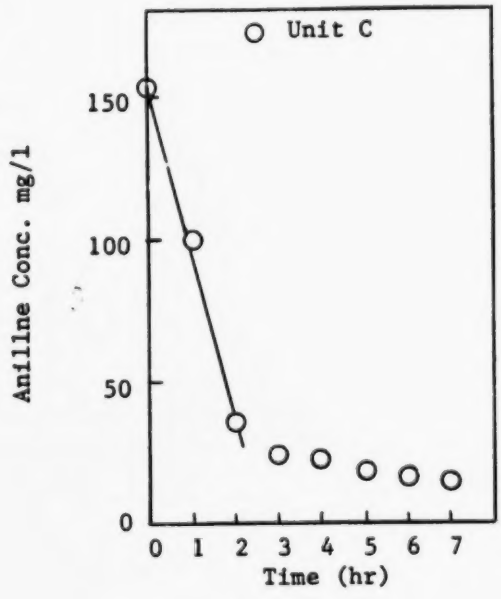


Figure A-7(c)

Aniline Removal

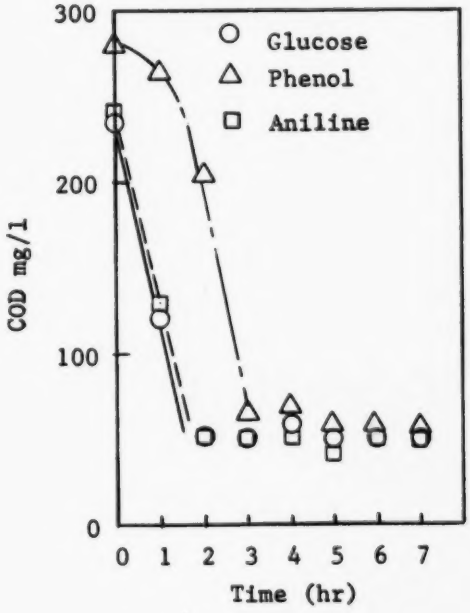


Figure A-7(d)

COD Removal - Single Substrates

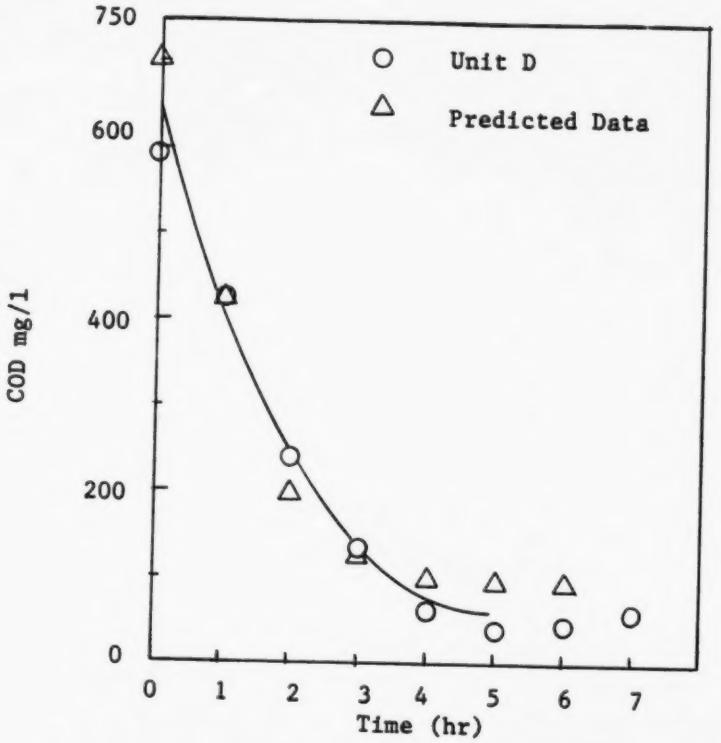


Figure A-7(e) COD Removal - Mixed Substrates

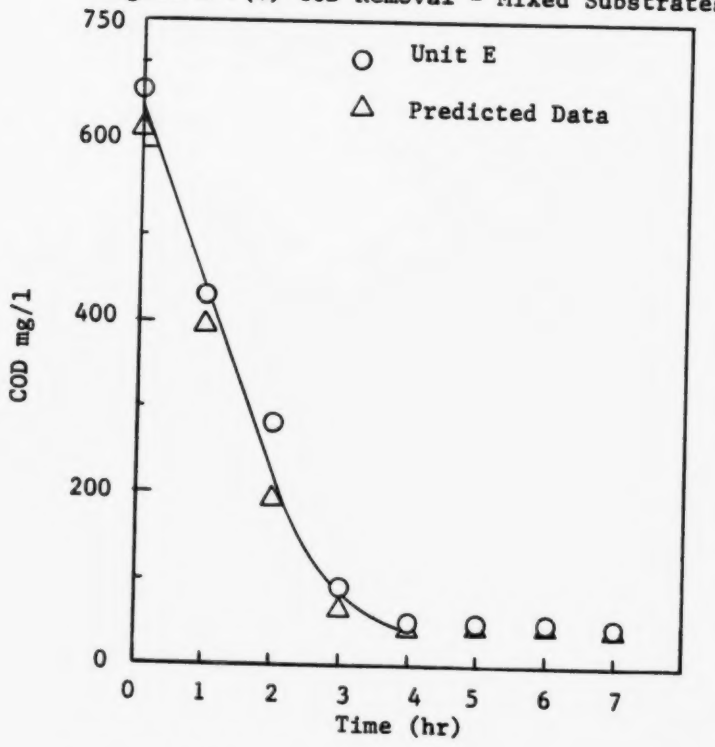


Figure A-7(f) COD Removal - Mixed Substrates

# APPENDIX A-8

## Batch Runs 5/18/67

**Continuous Unit Data:**

| Averages | Influent mg/l | Effluent mg/l | |
|---|---|---|---|
| COD | 910 | 45 | MLSS = 1930 |
| TOC | 561 | 26 | MLVSS = 1860 |
| Glucose | 300 | 6 | $L_f$ = 1.0 |
| Phenol | 150 | <1 | |
| Aniline | 150 | <1 | |

**Batch Data** (in mg/l unless otherwise indicated)

| Unit No. | Avg. MLVSS | Time (hr) | Glucose | Phenol | Aniline | COD | TOC | $BOD_5$ |
|---|---|---|---|---|---|---|---|---|
| A | | 0 | 280 | | | 340 | 122 | |
| | | 1 | 156 | | | 180 | 66 | |
| | | 2 | 24 | | | 53 | 19 | |
| | | 3 | 8 | | | 33 | 14 | |
| | 1941 | 4 | 6 | | | 45 | 12 | |
| | | 5 | 6 | | | 20 | 13 | |
| | | 6 | 6 | | | 20 | 18 | |
| | | 7 | 6 | | | 20 | 18 | |
| | | 24 | 8 | | | 20 | 17 | 6.6 |
| B | | 0 | | 93 | | 290 | 112 | |
| | | 1 | | 83 | | 310 | 108 | |
| | | 2 | | 70 | | 250 | 96 | |
| | | 3 | | 49 | | 170 | 64 | |
| | 1932 | 4 | | 20 | | 80 | 38 | |
| | | 5 | | <1 | | 40 | 14 | |
| | | 6 | | <1 | | 40 | 15 | |
| | | 7 | | <1 | | 40 | 15 | |
| | | 24 | | <1 | | 40 | 21 | 7.6 |
| C | | 0 | | | 130 | 340 | 108 | |
| | | 1 | | | 144 | 280 | 89 | |
| | | 2 | | | 124 | 200 | 61 | |
| | | 3 | | | 84 | 100 | 40 | |
| | 1985 | 4 | | | 45 | 50 | 21 | |
| | | 5 | | | 34 | 40 | 21 | |
| | | 6 | | | 29 | 50 | 19 | |
| | | 7 | | | 26 | 50 | 19 | |
| | | 24 | | | 22 | 50 | 20 | 10.8 |

APPENDIX A-8 (Contd.)

Batch Runs 5/18/67

Batch Data (Contd.)

| Unit No. | Avg. MLVSS | Time (hr) | Glucose | Phenol | Aniline | COD | TOC | $BOD_5$ |
|---|---|---|---|---|---|---|---|---|
| D | | 0 | 268 | 178 | 150 | 945 | 340 | |
| | | 1 | 184 | 162 | - | 745 | 272 | |
| | | 2 | 84 | 142 | - | 585 | 180 | |
| | | 3 | 10 | 100 | - | 400 | 128 | |
| | 2110 | 4 | 12 | 65 | - | 305 | 98 | |
| | | 5 | 13 | 37 | - | 210 | 68 | |
| | | 6 | 11 | 15 | - | 140 | 38 | |
| | | 7 | 8 | <1 | - | 53 | 20 | |
| | | 24 | 8 | <1 | - | 50 | 22 | |
| E | | 0 | 184 | 127 | 50 | 630 | 200 | |
| | | 1 | 96 | 100 | - | 475 | 146 | |
| | | 2 | 14 | 83 | - | 300 | 100 | |
| | | 3 | 16 | 65 | - | 250 | 79 | |
| | 1985 | 4 | 15 | 43 | - | 210 | 63 | |
| | | 5 | 10 | 7 | - | 84 | 31 | |
| | | 6 | 7 | <1 | - | 40 | 20 | |
| | | 7 | 8 | <1 | - | 40 | 16 | |
| | | 24 | 8 | <1 | - | 40 | 21 | |

BATCH DATA 5-18-67

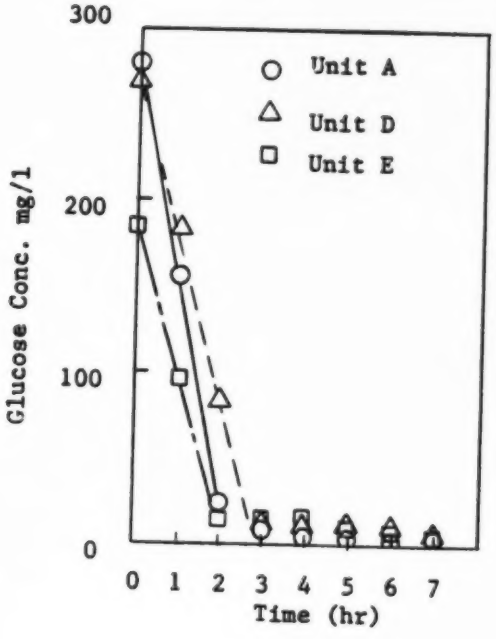


Figure A-8(a)

Glucose Removal

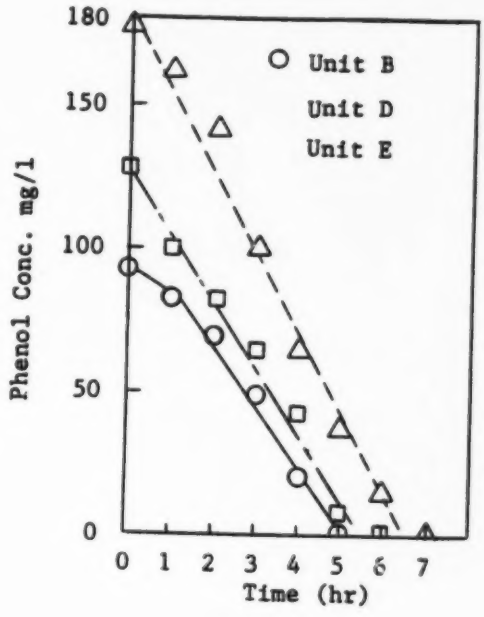


Figure A-8(b)

Phenol Removal

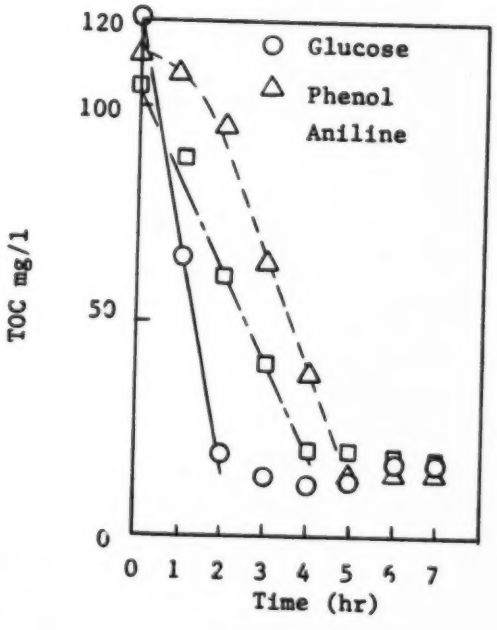


Figure A-8(c)

TOC Removal - Single Substrates

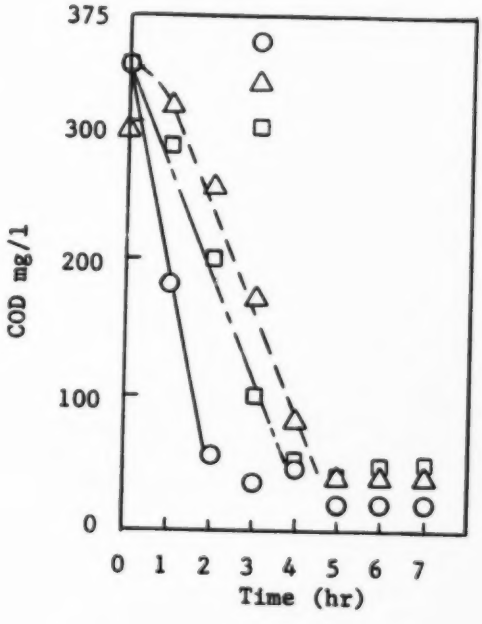


Figure A-8(d)

COD Removal - Single Substrates

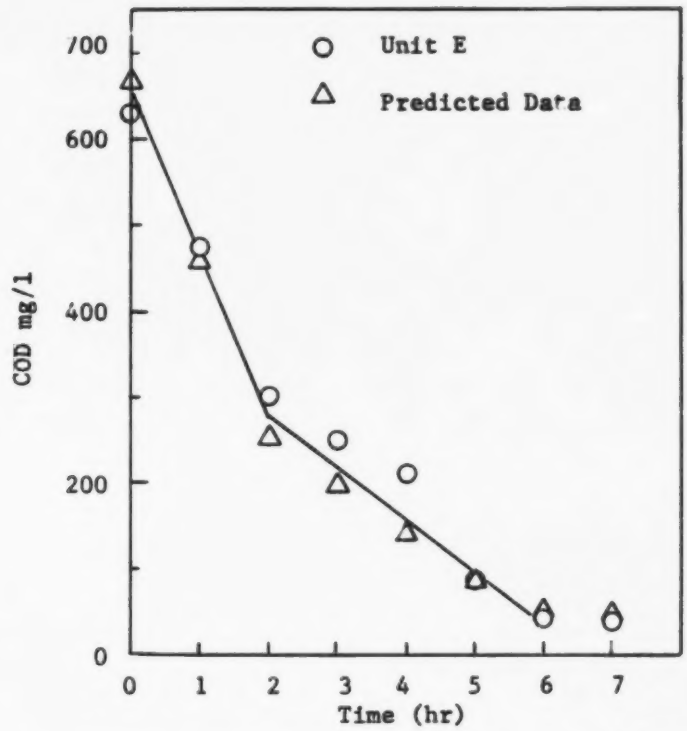


Figure A-8(e) COD Removal - Mixed Substrates

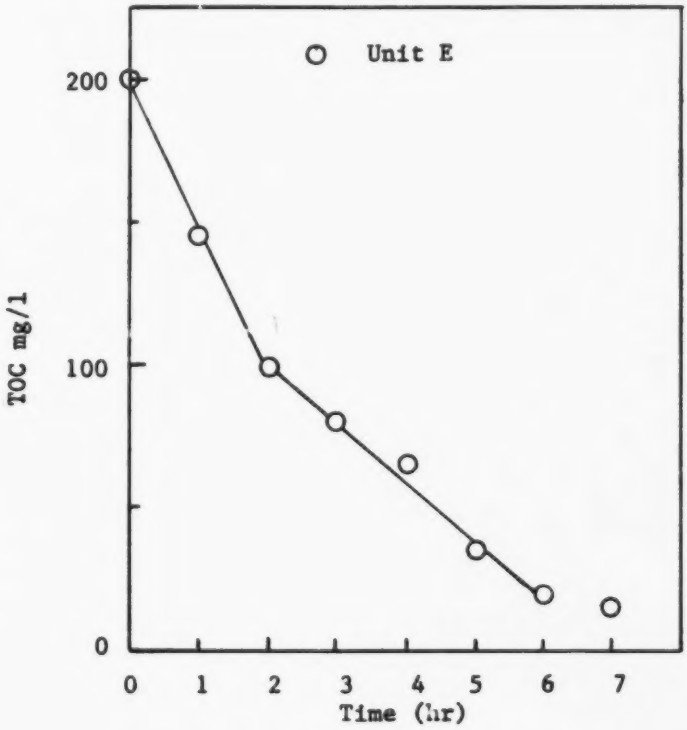


Figure A-8(f) TOC Removal - Mixed Substrates

# APPENDIX A-9

## Batch Runs 6/19/67

**Continuous Unit Data:**

| | Influent mg/l | Effluent mg/l | |
|---|---|---|---|
| Averages | | | |
| COD | 1280 | 150 | MLSS = 1250 |
| Glucose | 400 | 5 | MLVSS = 1140 |
| Phenol | 200 | 80 | $L_f$ = 3.4 |
| Aniline | 200 | 5 | |

**Batch Data** (in mg/l unless otherwise indicated)

| Unit No. | Avg. MLVSS | Time (hr) | Glucose | Phenol | Aniline | COD | TOC |
|---|---|---|---|---|---|---|---|
| A | | 0 | 386 | | | 565 | 216 |
| | | 1 | 300 | | | 505 | 178 |
| | | 2 | 250 | | | 480 | 150 |
| | | 3 | 184 | | | 390 | 126 |
| | 972 | 4 | 120 | | | 345 | 93 |
| | | 5 | 48 | | | 240 | 71 |
| | | 6 | 17 | | | 185 | 59 |
| | | 7 | 17 | | | - | 52 |
| | | 24 | 26 | | | 135 | 46 |
| B | 741 | 0 | | 260 | | 655 | 244 |
| | | 1 | | 245 | | 640 | 232 |
| | Toxic Effects | 2 | | 240 | | 605 | 244 |
| | Noted: | 3 | | 240 | | 670 | 238 |
| | Solids Level | 4 | | - | | 645 | 232 |
| | Dropping | 5 | | 240 | | 615 | 226 |
| | | 6 | | 245 | | 585 | 226 |
| | | 7 | | 195 | | 600 | 216 |
| | | 24 | | 162 | | 455 | 172 |
| C | | 0 | | | 184 | 675 | 208 |
| | | 1 | | | 166 | 635 | 212 |
| | | 2 | | | 138 | 570 | 188 |
| | | 3 | | | 64 | 510 | 176 |
| | 848 | 4 | | | - | 465 | 160 |
| | | 5 | | | - | 430 | 160 |
| | | 6 | | | 55 | 390 | 132 |
| | | 7 | | | 30 | 350 | 114 |
| | | 24 | | | 5 | 110 | 40 |

APPENDIX A-9 (Contd.)

Batch Runs 6/19/67

Batch Data (Contd.)

| Unit No. | Avg. MLVSS | Time (hr) | Glucose | Phenol | Aniline | COD | TOC |
|---|---|---|---|---|---|---|---|
| D | | 0 | 386 | 280 | 188 | 1530 | - |
| | | 1 | 328 | 265 | 184 | 1360 | 432 |
| | | 2 | 288 | 260 | 168 | 1370 | 424 |
| | | 3 | - | 255 | - | 1270 | 416 |
| | 858 | 4 | 216 | - | - | 1070 | 400 |
| | | 5 | 174 | 265 | - | 1030 | 400 |
| | | 6 | 72 | 255 | - | - | 358 |
| | | 7 | - | 250 | - | 1000 | 378 |
| | | 24 | 18 | 232 | 9 | 690 | 260 |
| E | | 0 | 186 | 185 | 170 | 1060 | 388 |
| | | 1 | 142 | 187 | 100 | 970 | 308 |
| | | 2 | 110 | - | - | 900 | 314 |
| | | 3 | 79 | - | - | 835 | 296 |
| | 878 | 4 | 50 | - | - | 800 | 262 |
| | | 5 | 24 | 165 | - | 720 | 244 |
| | | 6 | 12 | 167 | - | 680 | 100 |
| | | 7 | 13 | 152 | 73 | 680 | 100 |
| | | 24 | 16 | - | 10 | 420 | 112 |

BATCH DATA 6-19-67

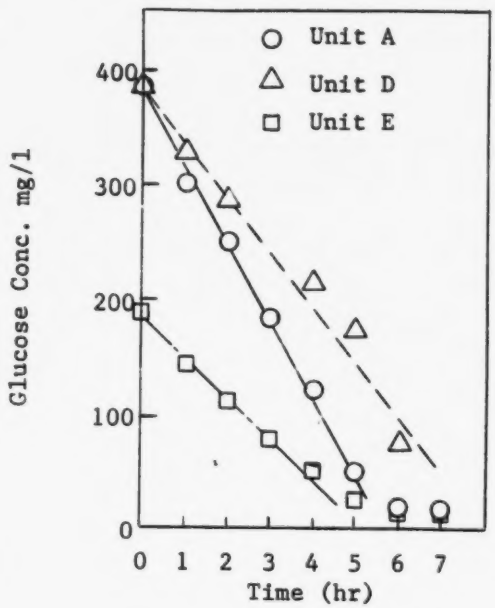


Figure A-9(a)

Glucose Removal

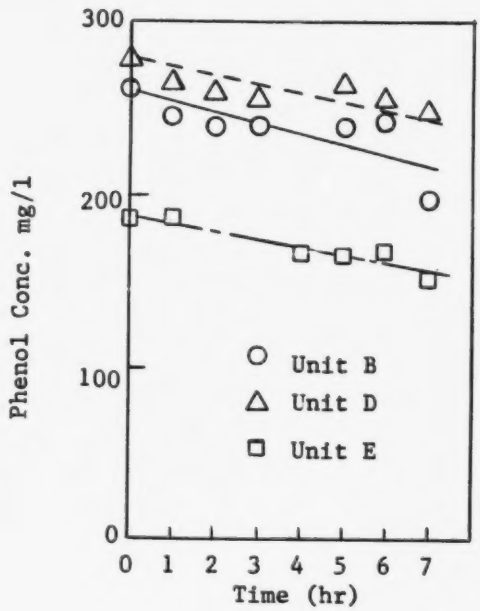


Figure A-9(b)

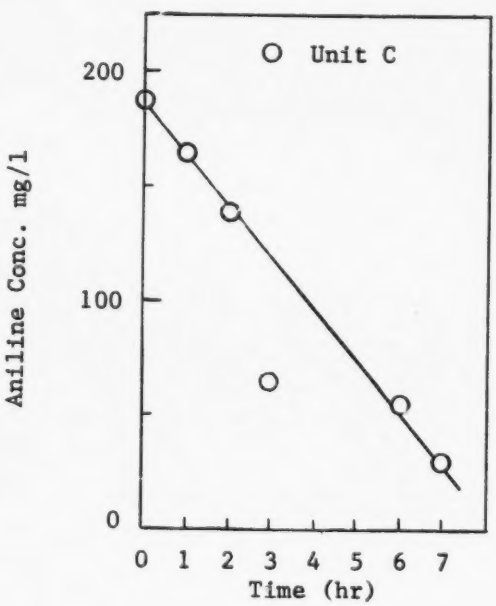


Figure A-9(c)

Aniline Removal

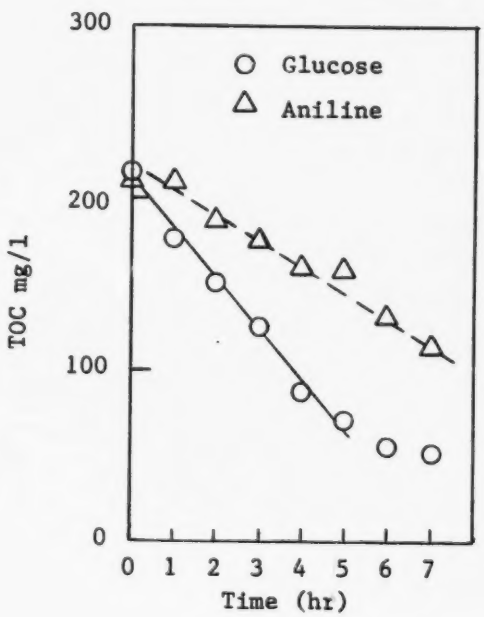


Figure A-9(d)

TOC Removal - Single Substrates

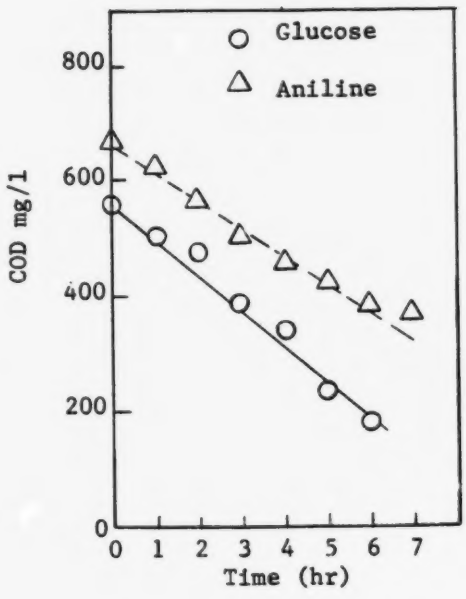


Figure A-9(e)

COD Removal - Single Substrates

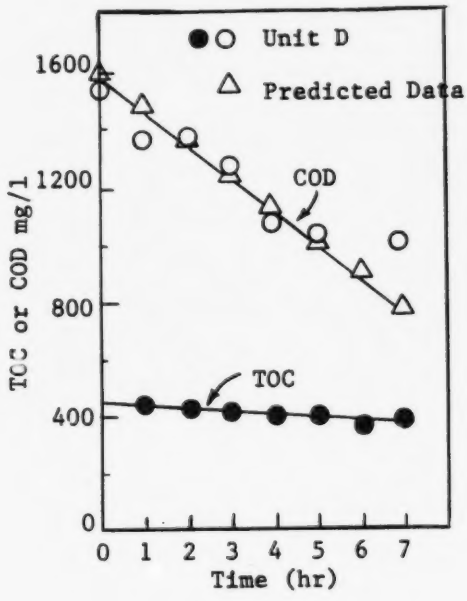


Figure A-9(f)

TOC and COD Removals
Mixed Substrates

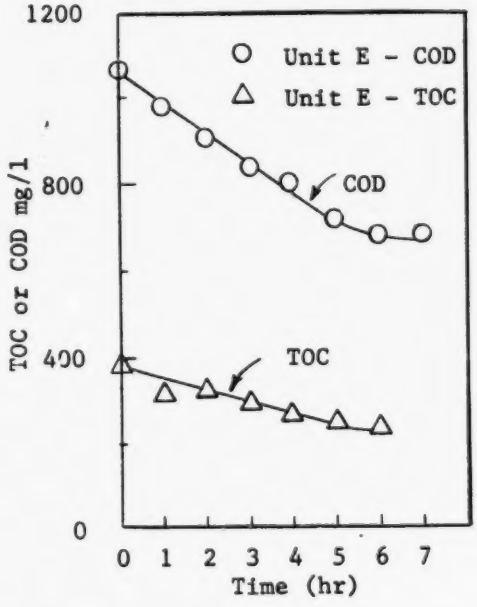


Figure A-9(g)

TOC and COD Removals
Mixed Substrates

# APPENDIX A-10

## Batch Runs 6/27/67

| Continuous Unit Data: | | Influent mg/l | Effluent mg/l | |
|---|---|---|---|---|
| Averages | | | | |
| | COD | 650 | 55 | |
| | TOC | - | 16 | |
| | Glucose | 200 | <1 | MLSS = 2730 |
| | Phenol | 100 | <1 | MLVSS = 2420 |
| | Aniline | 50 | <1 | $L_f$ = 0.65 |
| | Glycine | 200 | - | |
| | Sodium Acetate | 200 | - | |

Batch Data (in mg/l unless otherwise indicated)

| Unit No. | Avg. MLVSS | Time (hr) | Glucose | Phenol | Aniline | COD | TOC |
|---|---|---|---|---|---|---|---|
| A | | 0 | 172 | | | 280 | 90 |
| | | 1 | 98 | | | 180 | 56 |
| | | 2 | 39 | | | 120 | 32 |
| | | 3 | 8 | | | 80 | 20 |
| | 2330 | 4 | 12 | | | 80 | 22 |
| | | 5 | 7 | | | 60 | 19 |
| | | 6 | 6 | | | 60 | 20 |
| | | 7 | 7 | | | 60 | 16 |
| | | 24 | 7 | | | 80 | 20 |
| B | | 0 | | 56 | | 260 | 83 |
| | | 1 | | 51 | | 250 | 74 |
| | | 2 | | 42 | | 225 | 69 |
| | | 3 | | 34 | | 180 | 57 |
| | 2380 | 4 | | 14 | | 120 | 34 |
| | | 5 | | <1 | | 60 | 19 |
| | | 6 | | <1 | | 60 | 19 |
| | | 7 | | <1 | | 60 | 19 |
| | | 24 | | <1 | | 80 | 21 |
| C | | 0 | | | 92* | 190 | 54 |
| | | 1 | | | 72 | 145 | 43 |
| | | 2 | | | 50 | 90 | 32 |
| | | 3 | | | 22 | 50 | 21 |
| | 2300 | 4 | | | 21 | 60 | 22 |
| | | 5 | | | 16 | 50 | 21 |
| | | 6 | | | 15 | 60 | 22 |
| | | 7 | | | 12 | 60 | 20 |
| | | 24 | | | 11 | 80 | 21 |

*on U.V.

APPENDIX A-10 (Contd.)

Batch Runs 6/27/67

Batch Data (Contd.)

| Unit No. | Avg. MLVSS | Time (hr) | Glucose | Phenol | Aniline | COD | TOC |
|---|---|---|---|---|---|---|---|
| D | | 0 | 136 | 71 | 50 | 650 | 290 |
| | | 1 | 96 | 63 | - | 500 | 194 |
| | | 2 | 57 | 55 | - | 390 | 130 |
| | | 3 | 16 | - | - | 250 | 86 |
| | 2420 | 4 | 15 | 34 | - | 225 | 71 |
| | | 5 | 12 | 22 | - | 170 | 54 |
| | | 6 | 9 | 1 | - | 80 | 25 |
| | | 7 | 10 | <1 | - | 90 | 25 |
| | | 24 | 10 | <1 | - | 100 | 26 |
| | | | | Substrate | | | |
| E | | 0 | | 200 mg/l Glycine | | 145 | 74 |
| | | 1 | | | | 130 | 57 |
| | | 2 | | | | 115 | 37 |
| | | 3 | | | | 80 | 22 |
| | 2340 | 4 | | | | 90 | 22 |
| | | 5 | | | | 90 | 22 |
| | | 6 | | | | 90 | 22 |
| | | 7 | | | | 90 | 23 |
| | | 24 | | | | 90 | 22 |
| F | | 0 | | 200 mg/l Sodium | | 145 | 48 |
| | | 1 | | Acetate | | 90 | 20 |
| | | 2 | | | | 90 | 20 |
| | | 3 | | | | 80 | 20 |
| | 2340 | 4 | | | | 90 | 20 |
| | | 5 | | | | 90 | 20 |
| | | 6 | | | | 90 | 20 |
| | | 7 | | | | 90 | 22 |
| | | 24 | | | | 90 | 24 |

BATCH DATA 6-28-67

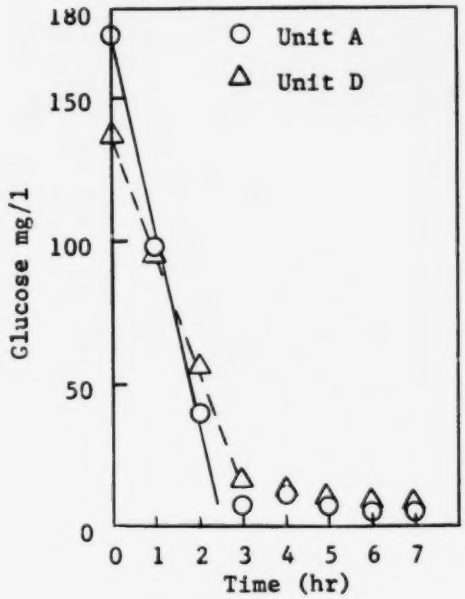


Figure A-10(a)

Glucose Removal

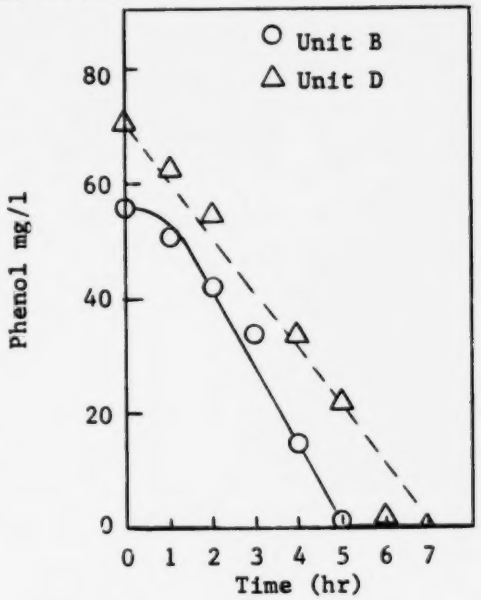


Figure A-10(b)

Phenol Removal

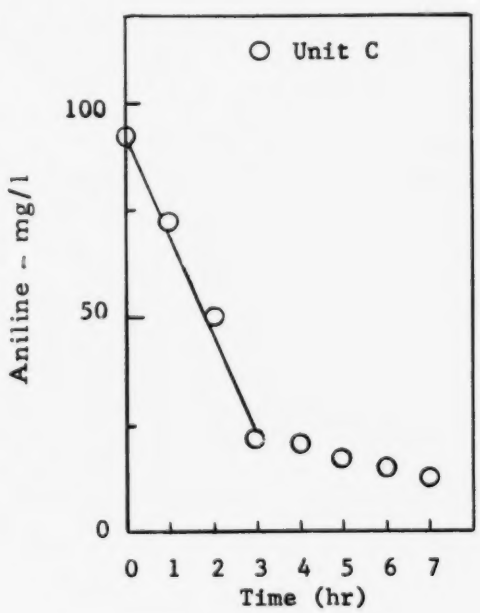


Figure A-10(c)

Aniline Removal

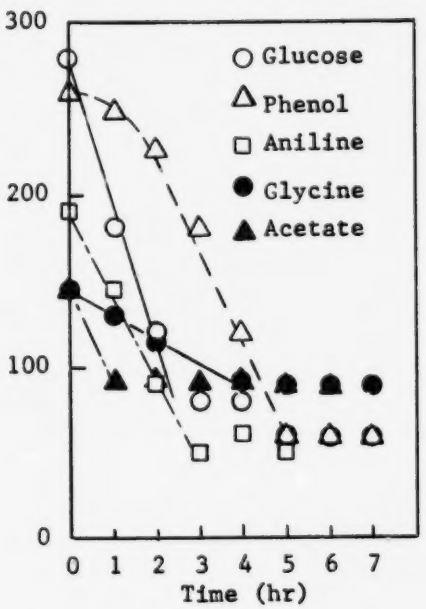


Figure A-10(d)

COD Removal
Mixed Substrates

# APPENDIX B

## B-1 TECHNICON AUTOANALYZER

The Technicon Autoanalyzer was used to measure the chemical oxygen demand of the samples to give an estimate of their organic content. The automated method is based on the reduction of orange $Cr^{+6}$ to green $Cr^{+3}$, which is measured on a flow-through colorimeter using a 420 mμ filter and recorded continuously on a strip chart.

The Autoanalyzer performs the COD test at the rate of ten samples per hour, thus materially reducing the analysis time for a large number of samples. The automatic sampler provided with the apparatus eliminates sampling error and the techniques of reagent addition and digestion are constant in the automated procedure.

The manual method (2) was used on random samples to check the accuracy of the Autoanalyzer. The manual and automated estimates of COD were compared, and the average percent error for nine selected samples was 4.6%.

A schematic of the COD analysis as run on the Autoanalyzer is shown in Figure B-1.

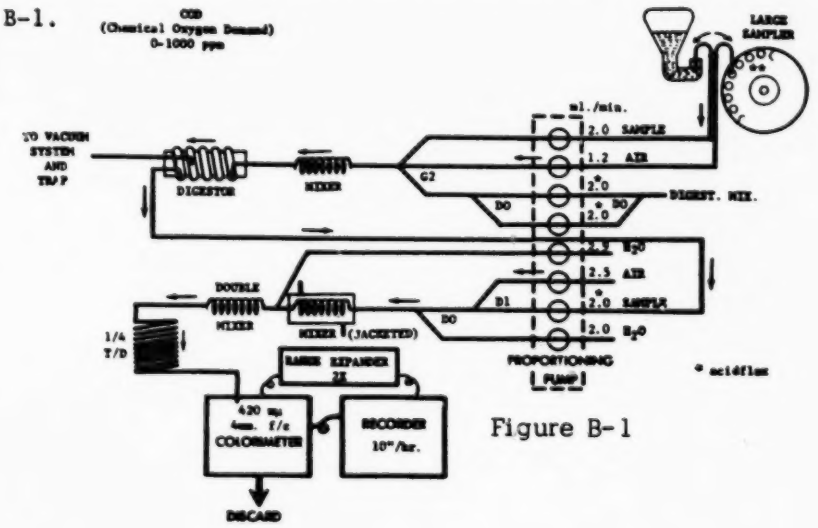


Figure B-1

## B-2 BECKMAN MODEL DB SPECTROPHOTOMETER

The Model DB spectrophotometer was used for all colorimetric determinations with the exception of COD. This instrument is a double-beam, ratio-recording spectrophotometer which uses a vibrating mirror assembly for alternate switching of the light beam from sample to reference solution (41). Monochromation of the light beam in this instrument is accomplished by means of a quartz prism. The spectrophotometer can be used in the range from 205 mu to 770 mu, that is, in both the ultraviolet and visible regions of the spectrum. Two light sources are used, depending on the wave length of light required. For operation in the ultraviolet range (205-380 mu), the hydrogen lamp is used. In the visible range (380-770 mu) a tungsten filament, incandescent lamp serves as the light source. It is necessary to use quartz cuvettes in the ultraviolet range.

The sample and reference solutions are placed in optically matched cuvettes which are then put in their respective positions in the spectrophotometer. The instrument reads the relative optical density or percent transmission of the sample as compared to the reference solution, on a deflection-type meter (41).

## B-3 BECKMAN ZEROMATIC II pH METER

All pH measurements were made with this instrument using the glass-calomel electrode system. This instrument is a temperature-compensated, direct-reading pH meter which uses an a-c amplifier (41). Prior to use, the meter was checked using a standard buffer solution near the pH range to be measured and was adjusted if necessary.

## B-4 OPTICAL MICROSCOPY

Slides of the activated sludge population were observed microscopically during each of the experimental runs. The slides were prepared by the Gram stain procedure and were observed through a binocular Bausch and Lomb microscope, using both the dry and oil immersion objectives.

Selected slides which best represented the microbial populations observed at the various organic loading levels were photographed using a Leitz "Orthomat" microscope camera and the Leitz "Ortholux" microscope. This microscope had a built-in illuminating system and used a fully-automatic 35 mm camera. Ektachrome-X film EX135-20 with an ASA rating of 64 was used for the photomicrographs.

## B-5 BECKMAN CARBONACEOUS ANALYZER (1)

This instrument operates on the principle that organic material will oxidize in the presence of pure oxygen gas, producing a given amount of carbon dioxide.

A hypodermic syringe is used to inject a very small amount of sample into the combustion tube of the instrument. The combustion tube, containing an asbestos packing coated with a cobalt oxide catalyst, is heated to approximately 950$^{o}$C and receives a constant stream of pure oxygen. Any organic material in the sample is completely oxidized to carbon dioxide in the combustion chamber.

The carbon dioxide formed is carried by the oxygen through an infrared analyzer. The signal from the infrared analyzer is proportional

to the carbon dioxide in the oxygen stream, and, therefore, to the carbon content of the sample (1). The reproducibility of data should be ±2% of the full-scale reading of the detector. Carbon dioxide-free acetic acid standards are used to calibrate the instrument. Maximum recommended scale range is 0-2000 mg/l of organic carbon.

In order to distinguish organic carbon from the dissolved carbon dioxide, bicarbonates, and carbonates which may be present in the waste sample, it is necessary to acidify the sample to below pH 1.5 and purge it with nitrogen gas to remove these compounds prior to injection into the Analyzer. If volatile organics are present in the sample, other means (1) must be used to distinguish organic carbon from carbonates and carbon dioxide.

# APPENDIX C

## C-1 GLUCOSE DETERMINATION (31)

The method used to measure glucose in the filtered samples was a modification of the anthrone method used by Miholits (31) and described in Snell and Snell (36).

The anthrone method is applicable to all carbohydrates. Various carbohydrate classes can be distinguished by the digestion time required for maximum color development (36). The only non-carbohydrate known to react with anthrone is furfural (31).

In this investigation glucose was the only carbohydrate used. Thus, this procedure provided a specific test for glucose with no interference from the other compounds used in the investigation.

Anthrone will react with glucose in a hot concentrated sulfuric acid solution to give a blue or green colored complex. The optical density of the colored solution is proportional to glucose concentration in the range 0-100 mg/l.

In order to obtain reproducible results from this technique, it is necessary to observe three precautions (31): (1) the anthrone reagent must be ice cold ( 2°C) when added to the sample; (2) the sample must be ice cold ( 2°C) when the reagent is added; and (3) a calibration curve should be prepared for each test, using a fresh standard solution each time.

Digestion time in the hot water bath of the mixed reagent and sample is also a variable. Since only one carbohydrate was present in the samples, and since standards were run with each group of samples,

time was not too critical in this analysis. A minimum digestion time of 15 minutes and a maximum of 25 minutes must be observed, however.

The calibration curve is prepared from a standard solution containing 100 mg/l of glucose. Standards containing 0.25 mg and 0.125 mg of glucose in 2.5 ml volumes plus a blank of 2.5 ml of distilled water are used to prepare the standard curve. These standards are treated in precisely the same manner as the samples.

Reagents

Anthrone Reagent--Determine the quantity of reagent required allowing 10 ml for each sample and standard. Dissolve 0.2 g of reagent grade anthrone per 100 ml of 95% sulfuric acid required. Store in refrigerator until cold, but not in excess of six hours.

95% Sulfuric Acid--Add 950 ml of concentrated sulfuric acid to 50 ml distilled water.

Standard Glucose Solution--100 mg/l anhydrous, reagent grade glucose (1 ml = 0.1 mg glucose).

Procedure

1) Add 2.5 ml of sample or diluted sample to a 15 x 75 mm test tube.

2) Add 2.5 ml of prepared standards to test tubes.

3) Add 2.5 ml distilled water to test tube (blank).

4) Place all tubes in ice water bath until cold.

5) Keeping the tubes in the ice water bath, rapidly add 10 ml chilled anthrone reagent to each sample with a syringe. Mix tubes vigorously and cap each with a glass marble.

6) Place all tubes in an 85°C hot water bath for 20 minutes.

7) Remove tubes from hot water bath and immediately place them in a cold water bath to stop the reaction.

8) Mix all tubes.

9) Store the tubes 24 hours at 4°C to obtain optimum color development (optional).

10) Mix all tubes.

11) Measure optical density or percent transmission of samples, standards, and blank at 620 mu with a spectrophotometer.

## C-2 ANILINE DETERMINATION (36)

A dilute solution of aniline will give a stable yellow color when mixed with calcium hypochlorite under strongly alkaline conditions. The only other compound which gives a similar color is o-toluidine (36).

This method is capable of measuring aniline with an accuracy of ±5% at concentrations below 60 mg/l. The minimum detectable concentration of aniline is about 0.5 mg/l.

A standard curve should be prepared each time the analysis is run. Standards containing 50 mg/l and 25 mg/l and a distilled water blank are used. The blank and standards are acidified and then treated exactly like the samples.

Reagents

1% Available Chlorine Solution--Shake 5 g of fresh calcium hypochlorite with 25 ml of distilled water for 10 minutes. Filter the solution into a bottle with a dropper and store.

10% Sodium Hydroxide Solution--Dissolve 10 g sodium hydroxide in distilled water and make up to 100 ml.

Procedure

1) Dilute 10 ml of acidified sample to 30 ml with distilled water.

2) Add 3 drops chlorine solution and mix.

3) After 2 minutes, add 1.5 ml of 10% NaOH solution and mix well.

4) Let stand 10 minutes.

5) Read sample against reagent blank at 465 mu on the spectrophotometer.

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